

and Periplocaceae (Judd et al., 1994; Civeyrel, 1996; Endress et al., 1996; Sennblad & Bremer, 1996; Sennblad et al., 1998), but in most of these studies many relevant taxa were not considered, and thus the monophyly of the Apocynaceae s.l. has not been adequately tested. The only comprehensive survey published to date that has included at least one taxon from all recognized tribes of the Apocynaceae s.l. is the doctoral thesis of Sennblad (1997) and the paper based thereon (Sennblad & Bremer, 2000). In their analysis, however, support values for many clades were very low, so that relationships among clades were poorly resolved. Moreover, the precise placements of the Asclepiadaceae and Periplocaceae clades are in conflict among existing phylogenetic trees. The phylogenetic reconstruction presented here includes representatives of all recognized tribes of the entire Apocynaceae clade, and with 152 taxa, is nearly double the number of taxa included in the Sennblad and Bremer (2000) analysis. In addition, our analysis is based on molecular as well as morphological data. Therefore it represents the most comprehensive phylogenetic investigation published to date on Apocynaceae s.l.

Our cladistic analysis of the Apocynaceae s.l. uses both molecular (*trnL* intron and *trnL-F* spacer) sequence and morphological propagule character data to estimate phylogenetic relationships within the family. One hundred and fifty-two accessions (113 genera, 146 species), representing all the major infrafamilial groups, are represented. Six morphological characters related to seed dispersal were combined with the molecular matrix. These morphological characters were included in order to generate hypotheses of character evolution and to examine how propagule-related features correlate with Asclepiadaceae and Periplocaceae clade diversification. A brief cladistic biogeographic interpretation of this group is finally presented.

MATERIALS AND METHODS

SPECIES SAMPLED AND SOURCES OF PLANT MATERIAL

Plant material used for DNA extraction was obtained from field, botanical garden, and herbarium sources (Appendix 1). Field- and garden-collected specimens were dried and stored in silica gel prior to DNA isolation. From herbarium specimens, ca. 0.5 cm² of plant tissue was removed and either stored in plastic tubes or used immediately. Plant parts used included leaves, flowers, and seeds.

DNA ISOLATION, SEQUENCING, AND ALIGNMENT

DNA was extracted according to two protocols: a silica milk method (Struwe et al., 1998) and a modified potassium ethyl xanthogenate (PEX)/urea procedure (after Jhingan, 1992). Both methods remove secondary compounds thought to interfere with PCR amplification. The silica milk extraction method is basically a scaled down version of the standard CTAB (hexadecyltrimethylammonium bromide) DNA extraction method (Doyle & Doyle, 1987), but the final step in DNA isolation and purification is based on DNA: silica affinity (Boom et al., 1990; Savolainen et al., 1995) and not precipitation.

The *trnL-F* region of the plastid genome was amplified using standard PCR parameters according to Taberlet et al. (1991). Bovine serum albumin (BSA, 0.04%) was included in the PCR recipe to facilitate the likelihood of PCR product. The PCR primers correspond to the forward "c" (CGAAATCGGTAGACGCTACG) and "e" (GGT-TCAAGTCCCTCTATCCC), and the reverse "d" (GGGGATAGAGGGACTTGAAC) and "f" (ATT-TGAACTGGTGACACGAG) primers (Taberlet et al., 1991). Every attempt was made to amplify the entire *trnL-F* region using primers "c" and "f," but when this was not possible, separate "c-d" and "e-f" amplifications were conducted. Amplification products were purified in a preparatory low-melt agarose gel (Fisher Biotech) and re-amplified prior to sequencing reactions. Cycle sequencing was performed with either fluorochrome-labeled or dRhodamine dideoxy chain terminators. The cycle sequencing products were cleaned using Princeton Separations Centri-Sep 100 columns (www.bioresearchonline.com, following the manufacturer's protocol) and evaluated with a Perkin Elmer Applied Biosystems ABI 377 automated sequencer and software. Sequence alignments for phylogenetic analysis were initially done in Sequencher 3.0 followed by visual estimation.

Forty-eight binary insertion/deletion characters were assigned to account for structural (insertion/deletion) information (Table 1). The *trnL* (UAA) 3' and ambiguous regions of sequence were excluded from maximum parsimony analysis and from the global alignment of 154 sequences (available on request).

MORPHOLOGICAL DATA

Fruit and seed characters related to dispersal mode (Table 2) were obtained from primary literature sources (denoted by * in the reference section),

Table 1. Sequence insertion/deletion characters that were scored from the total Apocynaceae s.l. *trnL* intron and *trnL*-F spacer alignment (available on request). The indel # lists the number and the range provides the exact location of the gap in the final alignment for the contiguous intron and spacer.

<i>trnL</i> intron				<i>trnL</i> -F spacer			
Indel #	Range (bp)	Indel #	Range (bp)	Indel #	Range (bp)	Indel #	Range (bp)
1	73 & 88	17	307–310	30	667–672	46	1049–1057
2	122–126/141–144	18	311–314	31	706–722	47	1114–1116
3	130–140	19	382–470	32	731–739	48	1189–1194
4	127–140	20	299–300	33	786–844		
5	135–140	21	301–302	34	824–1219		
6	134–140	22	293–302	35	873–878		
7	133–140	23	321–327	36	819		
8	152–157	24	344–367	37	838–843		
9	165	25	354–359	38	883–1044		
10	235–241	26	388	39	923–936		
11	233–253	27	425–438	40	924–936		
12	281–286	28	477–485	41	921–936		
13	291–300	29	500	42	982–991		
14	505–507			43	986–994		
15	531			44	1026–1047		
16	564–570			45	1053–1078		

as well as from personal observations. Interpretation of character states taken from the literature and/or from herbarium material was problematic given the state of the material and the lack of comprehensive propagule studies of the group as a whole. Since a large number of sources were consulted, character definitions were not consistent among these authors. Our morphological data set was, however, verified by consultation with M. Endress, J. Zarucchi, and W. D. Stevens. Definitions of characters and their states largely follow Endress et al. (1996).

PHYLOGENETIC ANALYSIS

The molecular data set and the combined data matrix (available upon request) were subjected to phylogenetic analysis. The molecular matrix consisted of the *trnL* intron, *trnL*-F spacer nucleotide sequence (see Table 3 for sequence characteristics), and binary insertion/deletion characters (Table 1). The combined matrix, however, consisted of both the molecular and the morphological propagule matrices so that considerations of morphological evolution could be made within the context of all available data (see Luckow & Bruneau, 1997). Each matrix was analyzed using Fitch parsimony (Fitch, 1971) as implemented in PAUP 4.0.0d64 (Swofford, 1998). The following options were used to find the shortest trees that were spread over several disjunct optima or islands: 100 replicates of random sequence addition order, saving no more

than 3 trees longer than an arbitrarily low “dummy” tree length per replicate, MULPARS activated, and TBR branch swapping. Following this initial search, the shortest trees obtained were amplified in number by swapping on trees in memory (using Nearest-Neighbor Interchange or NNI branch swapping) until a limit of 1000 trees was reached; only most parsimonious trees were saved. These tree amplifications were performed in order to approximate asymptotically all possible non-polytymous resolutions permitted by the data (Sanderson & Doyle, 1993). Strict consensus trees of the NNI-swapped trees were constructed to summarize components shared among all primary trees. Support for particular groups was evaluated using parsimony jackknife without character weights (Farris et al., 1996). One thousand jackknife replicates (“emulate Jac” on) with branch swapping (and 5 random addition orders per replicate) were performed using PAUP. Only groups with jackknife frequency of 50% or greater are considered to have support (see Farris et al., 1996); 63% or higher parsimony jackknife frequencies represent the equivalent of one uncontradicted synapomorphy (Farris et al., 1996; Struwe et al., 1998). Character evolution was studied with MacClade 3.0 (Maddison & Maddison, 1992). Character optimizations on strict consensus trees were made using the “hard polytomy” option, which assumes that every branch of a polytomy has acquired a character state independently from the others. For counts of numbers of particular char-

Table 2. Propagule character matrix for genera within Apocynaceae s.l. Propagule and geographic character optimization was performed but is not provided. Geographic distribution pertains to species or genera where applicable; this character was, however, not included in the actual phylogenetic analysis but is provided here for completeness.

	Exocarp	Mesocarp	Pericarp sclerifi- cation	Seed presenta- tion	Seed margin	Seed coma	Distribution
<i>Bonyunia superba</i>	0	0	2	0	1	1	0
<i>Strychnos tomentosa</i>	0	1	0	0&2	1&2	1	0
<i>Acokanthera oppositifolia</i>	0	1	0	2	2	1	1
<i>Acokanthera rotundata</i>	0	1	0	2	2	1	1
<i>Allamanda</i>	0	0	0	0	1	1	0
<i>Alstonia boonei</i>	0	0	0	0	2	0	1
<i>Alyxia buxifolia</i>	0	1	2	0	2	1	3
<i>Amsonia orientalis</i>	0	0	0	0	0&2	1	5
<i>Amsonia tabernaemontana</i>	0	0	0	0	2	1	6
<i>Apocynum androsaemifolium</i>	0	0	0	0	2	2	6
<i>Apocynum cannabinum</i>	0	0	0	0	2	2	6
<i>Asclepias curassavica</i>	0	0	0	0	1	2	0&7
<i>Asclepias syriaca</i>	0	0	0	0	1	2	6
<i>Asclepias tuberosa</i>	0	0	0	0	1	2	6
<i>Aspidosperma australe</i>	0	0	1&2	0	1	1	0
<i>Aspidosperma cuspa</i>	0	0	1&2	0	0	1	0
<i>Aspidosperma desmanthum</i>	0	0	1&2	0	1	1	0
<i>Aspidosperma megalocarpon</i>	0	0&1	2	0	1	1	0
<i>Aspidosperma myristicifolium</i>	0	0	1&2	0	1	1	0
<i>Aspidosperma pyriformis</i>	0	0	1&2	0	1	1	0
<i>Aspidosperma quebracho-blanco</i>	0	0	1&2	0	1	1	0
<i>Baisea leonensis</i>	0	0	0	0	2	2	1
<i>Beaumontia grandiflora</i>	0	0	1&2	0	2	2	0&2&4
<i>Bonaifousia siphilitica</i>	0	1	0	1	2	1	0
<i>Callichilia barteri</i>	0	1	0	1	2	1	1
<i>Calotropis procera</i>	0	0	0	0	1	2	2&5
<i>Caralluma crenulata</i>	0	0	0	0	1	2	1
<i>Caralluma gerstneri</i>	0	0	0	0	1	2	1
<i>Carissa bispinosa</i>	0	1	0	2	2	1	1
<i>Carissa carandas</i>	0	1	0	2	2	1	2
<i>Catharanthus roseus</i>	0	0	0	0	2	1	2
<i>Cerbera manghas</i>	1	0	1&2	0	0	1	3&4
<i>Cerbera odollam</i>	1	0	1&2	0	0	1	4
<i>Cerberiopsis candelabra</i>	1	0	1&2	0	0	1	4
<i>Ceropegia sandersoni</i>	0	0	0	0	1	2	1
<i>Chonemorpha fragrans</i>	0	0	0	0	2	2	2&3
<i>Condylocarpon amazonicum</i>	0	0	2	0	2	1	0
<i>Condylocarpon</i> sp.	0	0	2	0	2	1	0
<i>Couma macrocarpa</i>	0	1	0	2	2	1	0
<i>Cryptolepis oblongifolia</i>	0	0	0	0	2	2	1
<i>Cryptostegia grandiflora</i>	0	0	1&2	0	2	2	2
<i>Cycladenia humilis</i>	0	0	0	0	2	2	6
<i>Cynanchum</i> sp.	0	0	0	0	1	2	1&2&3&4&5&6
<i>Dischidia bengalensis</i>	0	0	0	0	1	2	4&5
<i>Ditassa</i> sp.	0	0	0	0	1	2	0
<i>Dregea sinensis</i>	0	0	1&2	0	1	2	5
<i>Duvalia elegans</i>	0	0	0	0	1	2	1
<i>Duvaliandra dioscoridis</i>	0	0	0	0	1	2	1
<i>Echidnopsis cereiformis</i>	0	0	0	0	1	2	1
<i>Echidnopsis dammanniana</i>	0	0	0	0	1	2	1
<i>Echites umbellata</i>	0	0	0	0	2	2	0&6&7
<i>Fockea cylindrica</i>	0	0	0	0	1	2	1
<i>Fockea edulis</i>	0	0	0	0	1	2	1

Table 2. Continued.

	Exocarp	Mesocarp	Pericarp sclerifi- cation	Seed presenta- tion	Seed margin	Seed coma	Distribution
<i>Fockea sinuata</i>	0	0	0	0	1	2	1
<i>Forsteronia corymbosa</i>	0	0	0	0	2	2	7
<i>Frerea indica</i>	0	0	0	0	1	2	2
<i>Funtumia elastica</i>	0	0	1&2	0	2	2	1
<i>Galactophora crassifolia</i>	0	0	0	0	2	2	0
<i>Geissospermum laeve</i>	0	1	0	2	2	1	0
<i>Gomphocarpus physocarpus</i>	0	0	0	0	2	2	1
<i>Gomphocarpus</i> sp.	0	0	0	0	2	2	1
<i>Gonolobus patens</i>	0	0	0	0	1	2	0&6
<i>Haplophyton crooksii</i>	0	0	0	0	2	2	0&6
<i>Himatanthus</i> sp.	0	0	0	0	1	1	0
<i>Himatanthus tarapotensis</i>	0	0	0	0	1	1	0
<i>Holarrhena pubescens</i>	0	0	0	0	2	2	1&2
<i>Hoya australis</i>	0	0	0	0	2	2	3&4
<i>Huernia keniensis</i>	0	0	0	0	1	2	1
<i>Hunteria umbellata</i>	0	1	0	2	2	1	1
<i>Isonema smeathmanni</i>	0	0	0	0	2	2	1
<i>Lacmellea aculeata</i>	0	1	0	2	2	1	0
<i>Landolphia incerta</i>	0	1	0	2	2	1	1
<i>Laxoplumeria baehniiana</i>	0	0	0	0	2	0	0
<i>Lepiniopsis ternatensis</i>	0	1	2	0	2	1	3&4
<i>Macoubea guianensis</i>	0	1	0	1	2	1	0
<i>Mandevilla</i> sp.	0	0	0	0	2	2	0
<i>Marsdenia amorimii</i>	0	0	0	0	1	2	0
<i>Mascarenhasia arborescens</i>	0	0	0	0	2	2	2
<i>Matelea</i> sp.	0	0	0	0	2	2	0&6
<i>Melodinus monogynus</i>	0	1	0	2	2	1	4
<i>Micholitzia obcordata</i>	0	0	0	0	1	2	2&4
<i>Microplumeria anomala</i>	0	0	0	0	2	1	0
<i>Molongum laxum</i>	0	1	0	2	2	1	0
<i>Mondia whiteii</i>	0	0	0	0	1	2	1
<i>Neisosperma nakiana</i>	0	1	2	0	0	1	4
<i>Nerium oleander</i>	0	0	0	0	2	2	1&2&5
<i>Ochrosia elliptica</i>	0	1	2	0	0	1	3&4
<i>Ophionella arcuata</i>	0	0	0	0	1	2	1
<i>Orbea variegata</i>	0	0	0	0	1	2	1
<i>Orbeanthus hardyi</i>	0	0	0	0	1	2	1
<i>Oxystelma bornouense</i>	0	0	0	0	2	2	1
<i>Pachycarpus</i> sp.	0	0	0	0	1	2	1
<i>Pacouria guianensis</i>	0	1	0	2	2	1	0
<i>Parsonsia eucalyptophylla</i>	0	0	0	0	2	2	3
<i>Peltastes peltatus</i>	0	0	0	0	2	2	0
<i>Pentalinon luteum</i>	0	0	0	0	2	2	7
<i>Periploca graeca</i>	0	0	0	0	2	2	5
<i>Petopentia natalensis</i>	0	0	0	0	2	2	1
<i>Philibertia gilliesii</i>	0	0	0	0	1	2	0
<i>Piaranthus geminatus</i>	0	0	0	0	1	2	1
<i>Picalima nitida</i>	0	1	0	2	2	1	1
<i>Plectaneia stenophylla</i>	0	0	0	0	0	1	2
<i>Pleiocarpa mutica</i>	0	1	0	2	2	1	1
<i>Plumeria alba</i>	0	0	0	0	0	1	7
<i>Plumeria obtusa</i>	0	0	0	0	0	1	0&7
<i>Prestonia mollis</i>	0	0	0	0	2	2	0
<i>Prestonia quinqueangularis</i>	0	0	0	0	2	2	0&7
<i>Pteralyxia kauaiensis</i>	0	1	2	0	2	1	4

Table 2. Continued.

	Exocarp	Mesocarp	Pericarp sclerifi- cation	Seed presenta- tion	Seed margin	Seed coma	Distribution
<i>Rauwolfia balansae</i>	0	1	2	0	2	1	4
<i>Rauwolfia serpentina</i>	0	1	2	0	2	1	2&4
<i>Saba comorensis</i>	0	1	0	2	2	1	1&2
<i>Sarcostemma mulanjense</i>	0	0	0	0	1	2	1&2
<i>Sarcostemma stolonifera</i>	0	0	0	0	1	2	1&2
<i>Secamone glaberrima</i>	0	0	0	0	2	2	2
<i>Secamone oleaeifolia</i>	0	0	0	0	2	2	2
<i>Skytanthus acutus</i>	0	0	1&2	0	2	1	0
<i>Stapelia leendertziae</i>	0	0	0	0	1	2	1
<i>Stapelia peglere</i>	0	0	0	0	1	2	1
<i>Stapelia revoluta</i>	0	0	0	0	1	2	1
<i>Stapelia schinzii</i>	0	0	0	0	1	2	1
<i>Stomatostemma monteiroae</i>	0	0	0	0	2	2	1
<i>Strepeliopsis strepelioides</i>	0	0	0	0	0	1	7
<i>Strophanthus gratus</i>	0	0	1&2	0	2	2	1
<i>Tabernaemontana citrifolia</i>	0	1	0	1	2	1	7
<i>Tabernaemontana floribunda</i>	0	1	0	1	2	1	3&4
<i>Tabernaemontana</i> sp.	0	1	0	1	2	1	2&4&7
<i>Tabernanthe iboga</i>	0	1	0	1	2	1	1
<i>Tavaresia grandiflora</i>	0	0	0	0	1	2	1
<i>Telosma cordata</i>	0	0	1&2	0	1	2	4
<i>Thevetia ahouai</i>	0	1	2	0	0	1	0
<i>Thevetia peruviana</i>	1	0	1&2	0	0	1	0
<i>Tonduzia longifolia</i>	0	0	0	0	0	0	0
<i>Tonduzia stenophylla</i>	0	0	0	0	0	0	0
<i>Trachelospermum jasminoides</i>	0	0	0	0	2	2	4&5
<i>Trichocaulon triebneri</i>	0	0	0	0	1	2	1
<i>Tridentea longipes</i>	0	0	0	0	1	2	1
<i>Tromotriche revoluta</i>	0	0	0	0	1	2	1
<i>Tweedia coerulea</i>	0	0	0	0	1	2	0
<i>Tylophora sylvatica</i>	0	0	0	0	1	2	1&2
<i>Urceola rosea</i>	0	0	0	0	2	2	2&4
<i>Vallesia antillana</i>	0	1	2	0	2	1	6&7
<i>Vinca difformis</i>	0	0	0	0	2	1	5
<i>Vinca minor</i>	0	0	0	0	2	1	5
<i>Vincetoxicum hirundinaria</i>	0	0	0	0	1	2	5
<i>Vincetoxicum nigrum</i>	0	0	0	0	1	2	5
<i>Voacanga africana</i>	0	1	0	1	2	1	1
<i>Wrightia tomentosa</i>	0	0	0	0	2	2	2&4

Characters & States

Exocarp

0: thin

1: thick

Mesocarp

0: dry

1: fleshy

Pericarp sclerification

0: non-sclerified

1: mesocarp

2: endocarp

Seed presentation

0: naked

1: arillate

2: embedded in pulp

Seed margin

0: not circumalate

1: circumalate

2: naked

Seed coma

0: ill defined

1: absent

2: well defined

Distribution

0: South-Meso America

1: Africa

2: Indian Ocean Basin

3: Australia-New Guinea

4: Pacific

5: temperate Eurasia

6: North America

7: Caribbean

Table 3. Sequence characteristics of the plastic *trnL* intron and *trnL*-F spacer sequence for members of the Apocynaceae s.l.

Sequence characteristic	<i>trnL</i> intron	<i>trnL</i> -F spacer
Nucleotide sites (bp)		
Total length (without gaps)	range (348–496) mean = 481	range (246–378) mean = 322
No. aligned positions (with gaps)	625	610
No. (%) ambiguous	58 (9.3)	164 (26.9)
No. (%) constant	319 (51)	214 (35.1)
No. (%) autapomorphic	95 (15.2)	75 (12.3)
No. (%) phylogenetically informative	153 (24.5)	157 (25.7)
Length variation (bp)		
No. of unambiguous alignment gaps	51	52
No. (size range) of deletions	13 (1–89)	11 (6–396)
No. (size range) of insertions	16 (1–21)	8 (1–14)
No. of gaps phylogenetically informative	29	19
No. (%) of point gaps	13224 (15.1)	19000 (27.7)
Sequence divergence (%)	0.2–12.2	0–17.6
G + C (%) content (range, mean)	range (35.9–39.9) mean = 36.9	range (33.9–37.8) mean = 35.3
Ts/Tv ratio (range)	1.27–1.28	1.07–1.08

acter state transformations, only unambiguous events were included (excluding outgroup taxa).

RESULTS AND DISCUSSION

MOLECULAR AND COMBINED MOLECULAR–MORPHOLOGICAL PHYLOGENY

Parsimony analysis of the molecular and combined matrix yielded **132**/120 most parsimonious trees of length **1218**/1294 steps (including autapomorphies and uninformative characters) and ensemble consistency (CI) and retention (RI) indices (Farris, 1989) of **0.53**/0.52 and **0.89**/0.89, respectively. A strict consensus was computed after the **132**/120 initial trees were amplified to a maximum of 1000 (Fig. 1). Values that pertain to the analysis of the molecular data (number of trees, CIs, RIs, jackknife support) always appear first in bold; those that apply to analysis of the combined data follow.

The phylogenies derived from the molecular and combined data are similar in both strict consensus structure and jackknife support. A single incongruency involves a weak association between *Rauvolfia* and *Tonduzia* (**55**/ $<50\%$). Other variations involve slight differences in tree resolution (Figs. 1, 2, indicated by dashed nodes)—the strict consensus of the molecular matrix is slightly less resolved than that for the combined analysis. The increased resolution obtained by combining the

molecular and morphological data further supports the usefulness of including morphological characters in this analysis. As would be expected, the addition of the few representative morphological characters did not significantly influence parsimony jackknife support, except for the *Neisosperma/Ochrosia* clade where support increased from <50 to 73%. The other sizable difference in support is seen at the very base of the tree for the genus *Aspidosperma* (<50 /63). These large changes in support argue for the continued recognition of *Neisosperma* and *Ochrosia* and perhaps the re-examination of circumscriptions for these genera (see Leeuwenberg (1994) for difference of opinion, and Potgieter (1999) for the examination of the genus *Aspidosperma*).

Parsimony jackknife values demonstrate strong support for the basic internal structure of the tree. Notably, (1) the monophyly of Apocynaceae s.l. is supported by **100**/100% of the jackknife replicates: similar branching order was seen for the analysis of a small number (11) of representative members of Apocynaceae using *trnL* spacer sequences together with Gentianaceae and Rubiaceae as outgroups (Struwe et al., 1998); (2) the genus *Aspidosperma* and its allies are sister to the rest of Apocynaceae in **94**/96% of replicates; (3) the genus *Alstonia* s. str. (i.e., excluding *Tonduzia*), in turn, is the sister group to the rest of the family at **95**/97%; (4) the fleshy-fruited genera *Acokanthera*

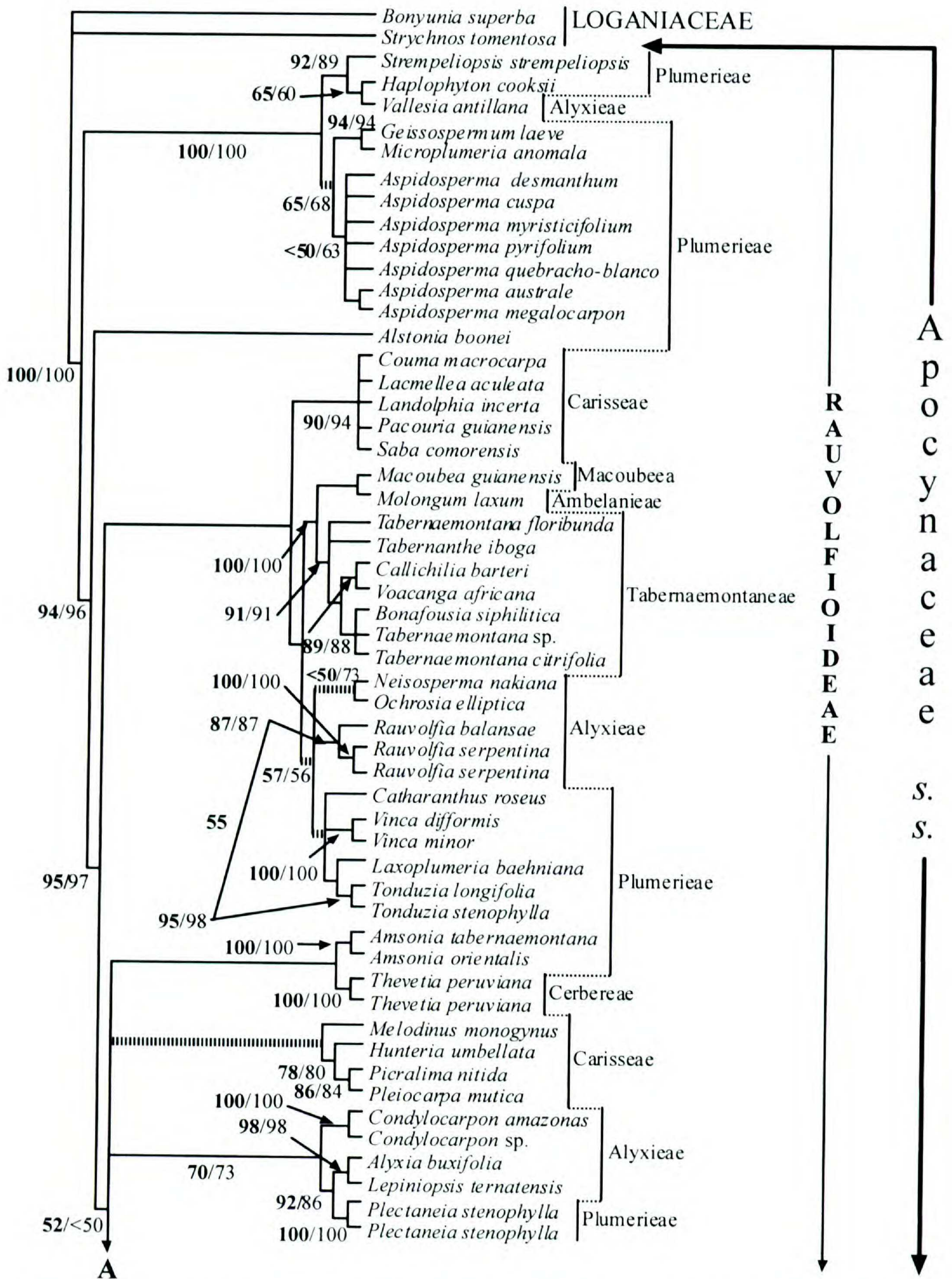
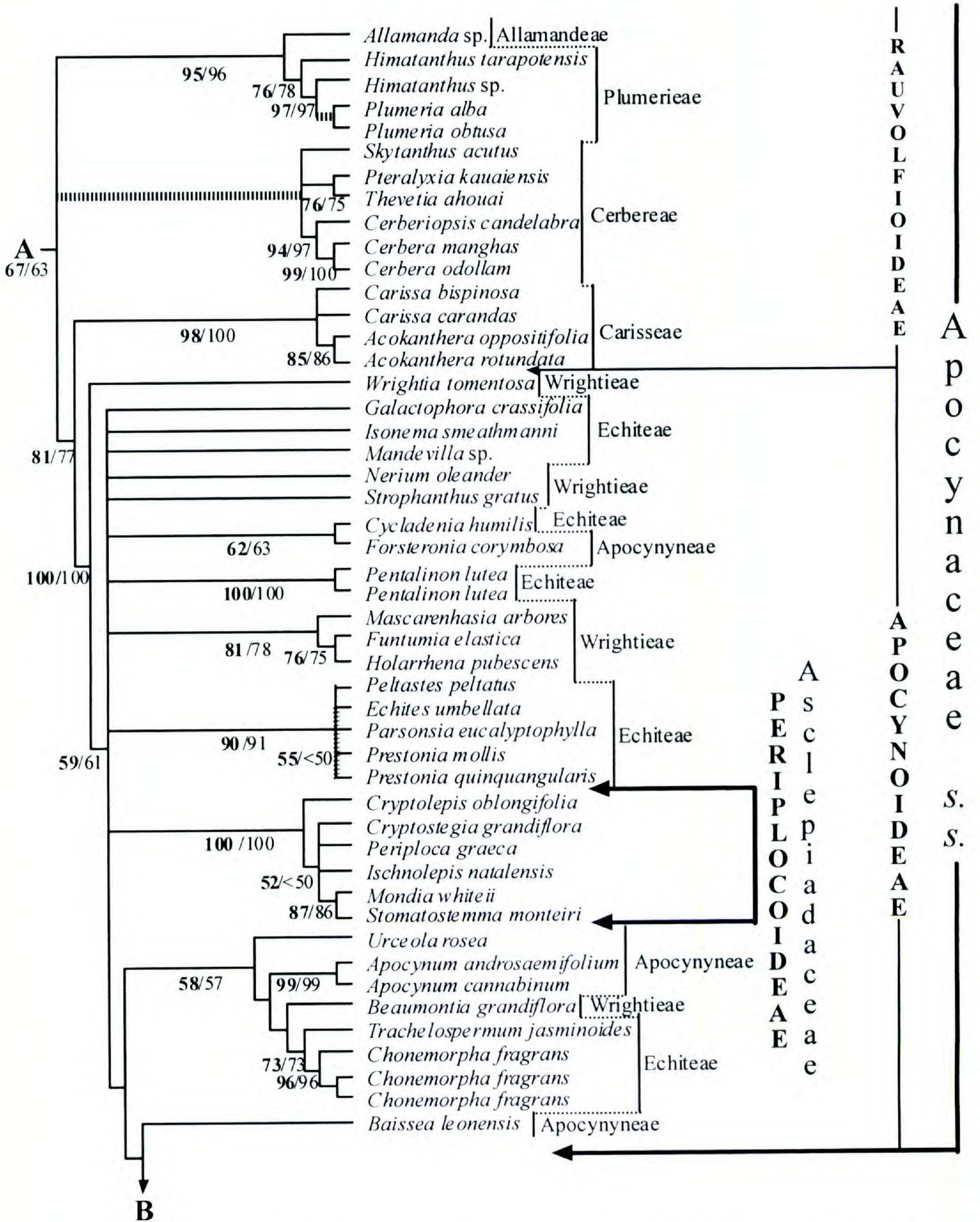


Figure 1 (pp. 530–532). Strict consensus of 1000 equally parsimonious trees (1218/1294 steps) for the molecular (*trnL* intron and *trnL-F* spacer nucleotide) matrix and the combined molecular and morphological (six propagule characters) data sets. The separate matrices are available from the first author upon request. Jackknife support values are indicated as the first value below the nodes for the tree generated from the molecular data alone—the second value represents jackknife support for the combined data matrix; black arrows denote support for a given node when space was limited. Dashed nodes are used to indicate additional structure seen in the combined data set, e.g., [(*Neisosperma*, *Ochrosia*), (*Catharanthus*–*Tonduzia*), (*Melodinus*–*Pleiocarpa*), (*Plumeria*), (*Skytanthus*–*Cerbera*), and the *Gomphocarpus*



clade], whereas dashed vertical lines indicate additional structure in the molecular data set [(*Peltastes*–*Prestonia*), (*Marsdenia*, *Telosma*)]. The only structural dissimilarity occurred in a relatively unsupported portion of the tree in the molecular clade consisting of [*Rauwolfia* (*Laxoplumeria*, *Tonduzia*)]. Consistency (CI) and retention (RI) indices for the molecular data set and the combined data set were **0.53/0.52** and **0.89/0.89**, respectively. The subfamilial and tribal classifications sensu Leeuwenberg (1994) and Swarupnandan et al. (1996) are compared with this phylogenetic tree.

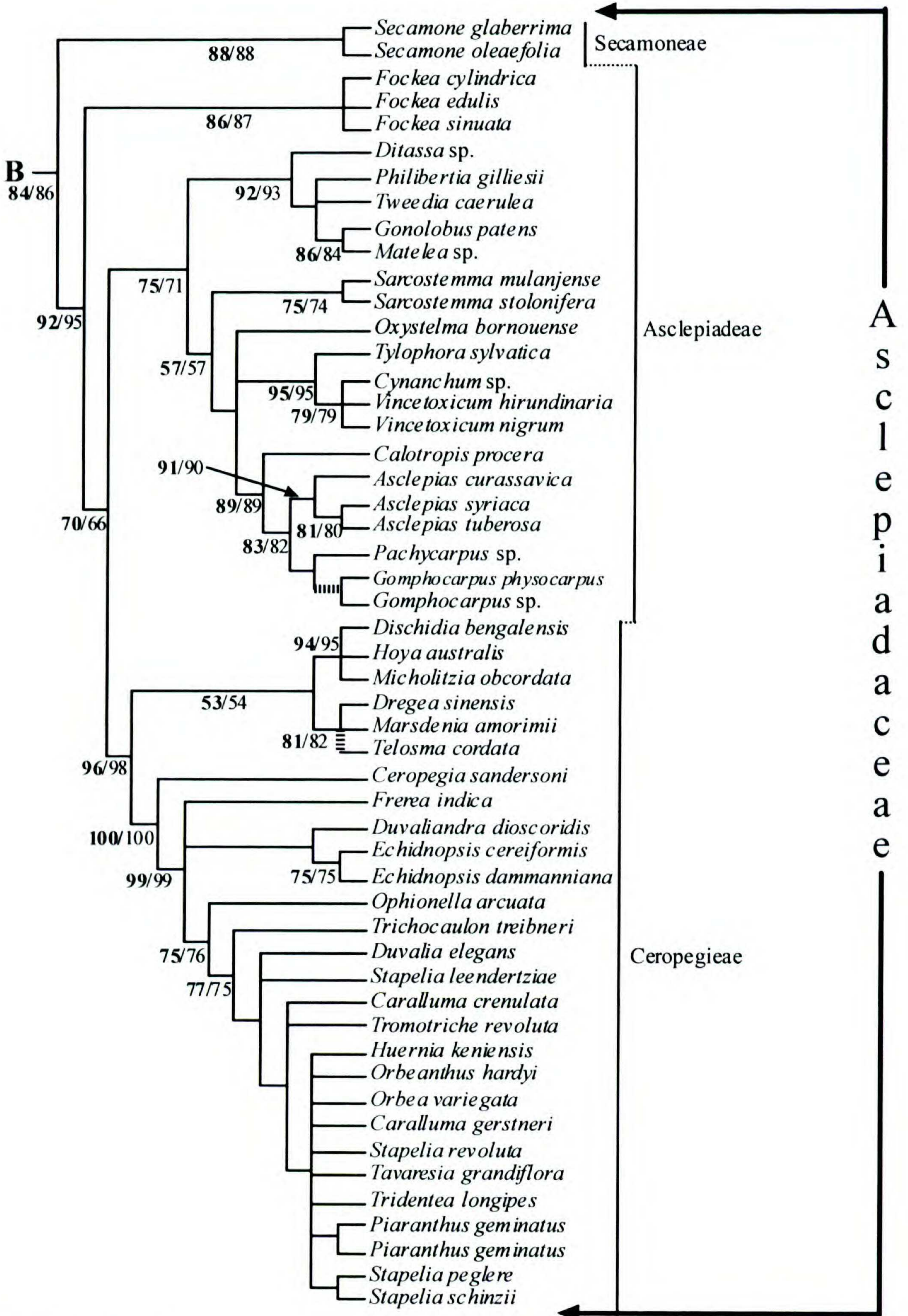


Figure 1. Continued.

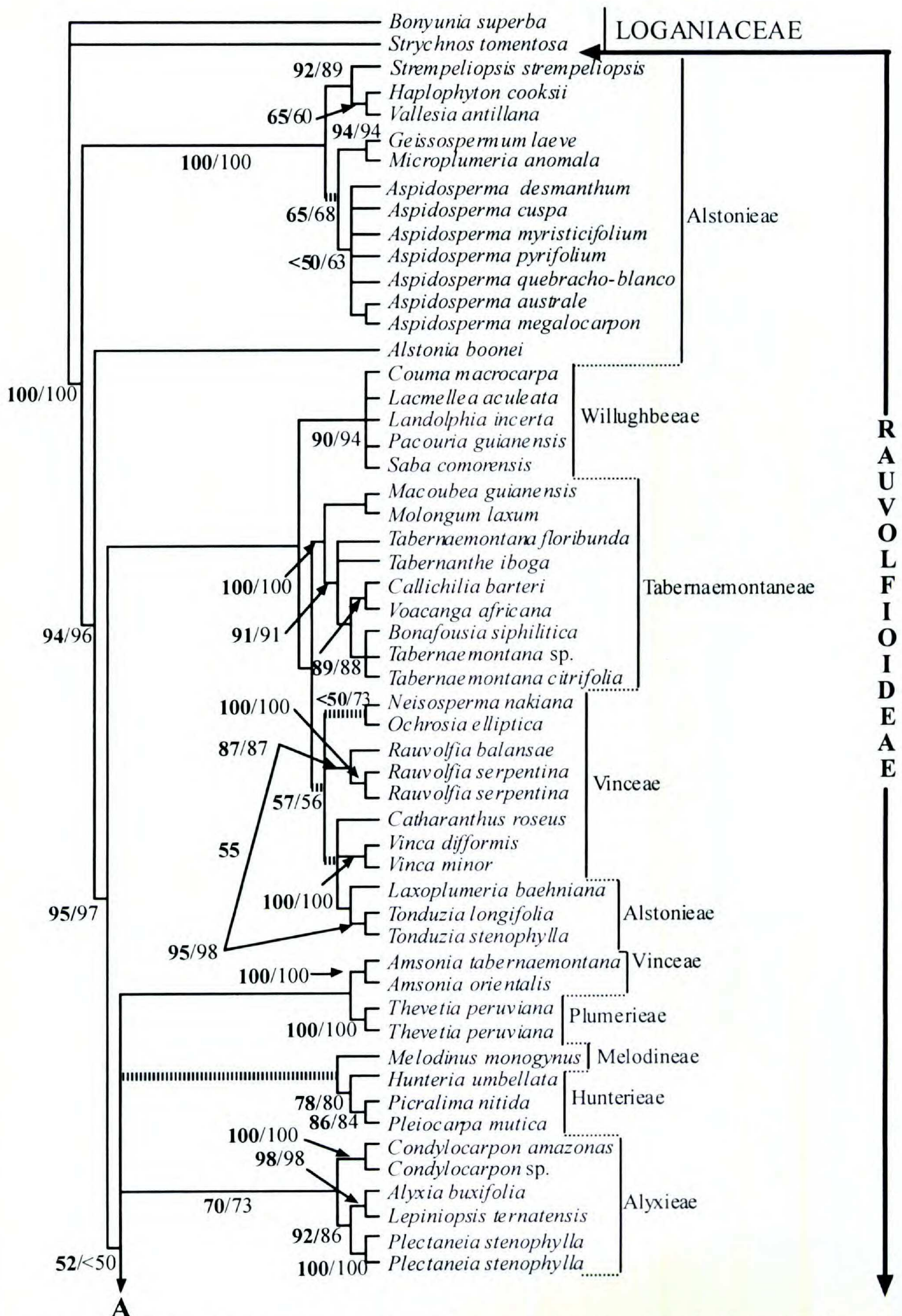
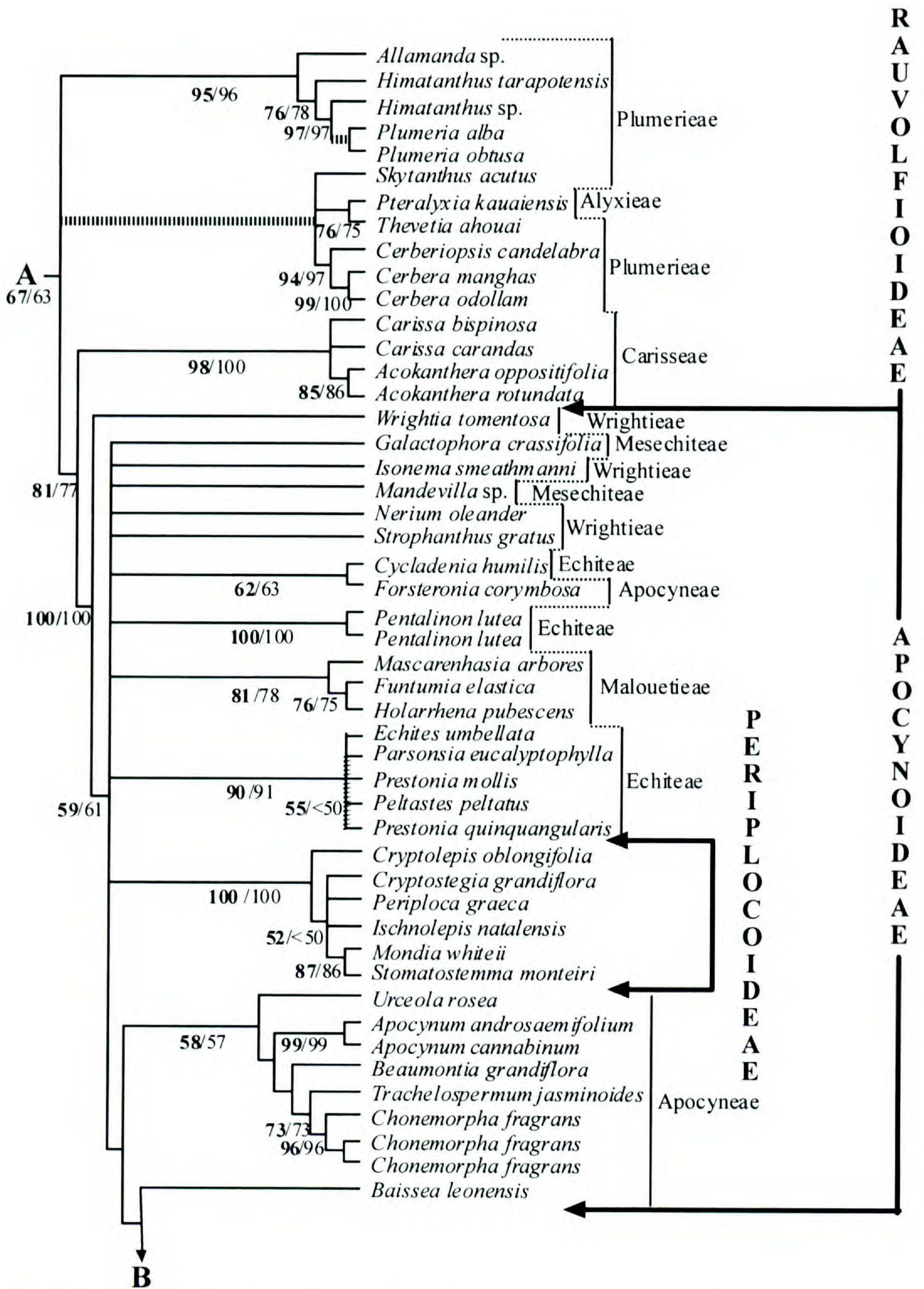


Figure 2 (pp. 533–535). Strict consensus of 1000 equally parsimonious trees (1218/1294 steps) for the molecular (*trnL* intron and *trnL*-F spacer nucleotide) matrix and the combined molecular and morphological (six propagule characters) data sets. The separate matrices are available from the first author upon request. Jackknife support values are indicated as the first value below the nodes for the tree generated from the molecular data alone—the second value represents jackknife support for the combined data matrix; black arrows denote support for a given node when space was limited. Dashed nodes are used to indicate additional structure seen in the combined data set, e.g., [(*Neisosperma*, *Ochrosia*), (*Catharanthus*–*Tonduzia*), (*Melodinus*–*Pleiocarpa*), (*Plumeria*), (*Skytanthus*–*Cerbera*), and the *Gomphocarpus*



clade] whereas dashed vertical lines indicate additional structure in the molecular data set [(*Peltastes*–*Prestonia*), (*Marsdenia*, *Telosma*)]. The only structural dissimilarity occurred in a relatively unsupported portion of the tree in the molecular clade consisting of [*Rauwolfia* (*Laxoplumeria*, *Tonduzia*)]. Consistency (CI) and retention (RI) indices for the molecular data set and the combined data set were **0.53/0.52** and **0.89/0.89**, respectively. The subfamilial and tribal classification sensu Endress and Bruyns (2000) is compared with this phylogenetic tree.

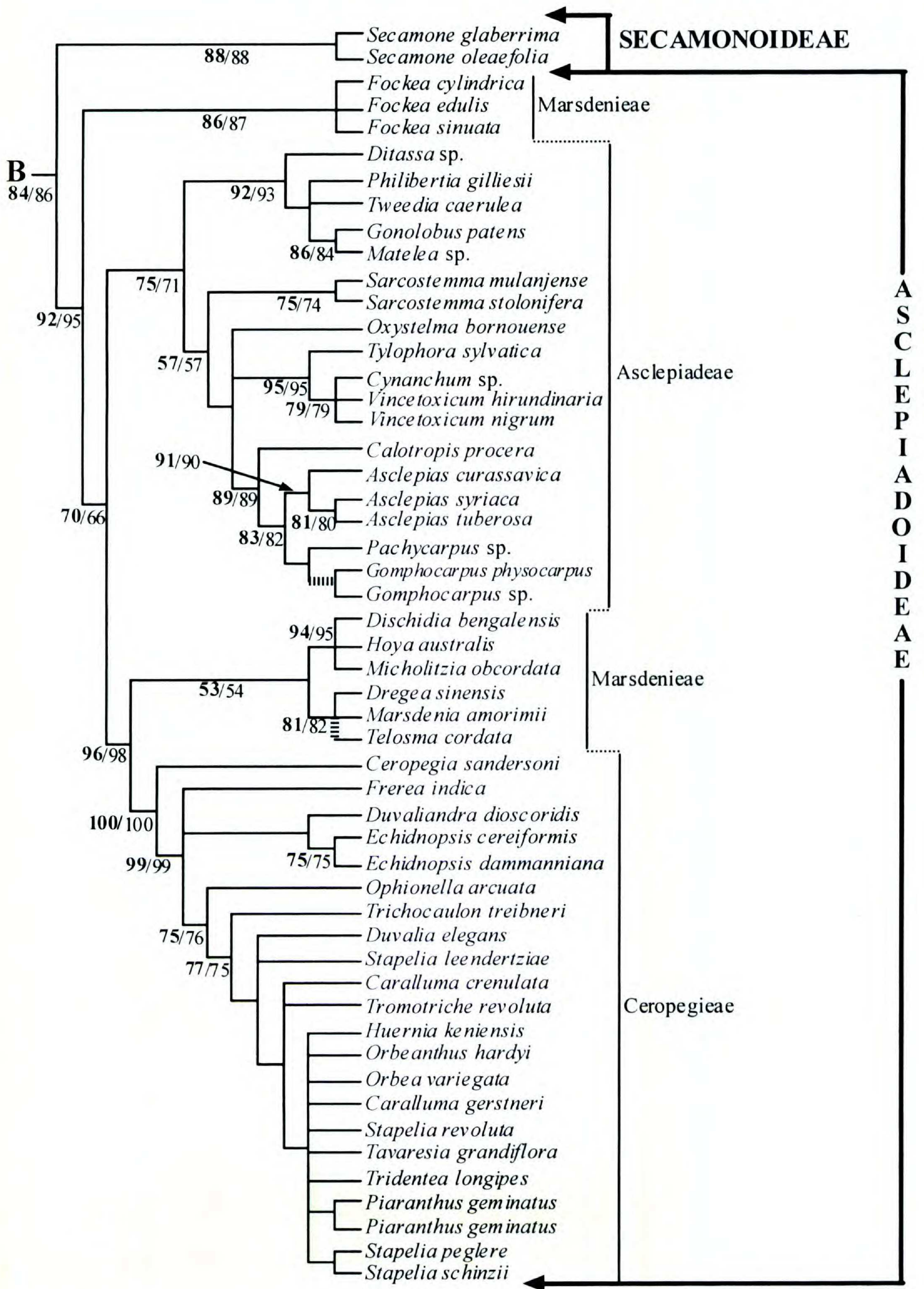


Figure 2. Continued.

and *Carissa* are resolved as sister genera to the “higher” Apocynaceae, Periplocaceae, and Asclepiadaceae in **81/77%** of replicates; and (5) the Apocynoideae, Periplocaceae, and Asclepiadaceae-clade is supported as monophyletic in **100/100%** of jackknife replicates, as are the Periplocaceae and Asclepiadaceae themselves (**100/100%** and **84/86%** of jackknife replicates, respectively).

THE APOCYNACEAE SENSU STRICTO ARE
PARAPHYLETIC TO THE ASCLEPIADACEAE AND THE
PERILOCACEAE

The non-monophyly of the traditionally maintained Apocynaceae s. str., with respect to the Periplocaceae and the Asclepiadaceae, is increasingly being recognized. For example, MacFarlane's (1933: 15) treatise on the evolution and distribution of both families included the explicit statement that “the extremely complex and highly evolved flower of Asclepiadaceae can be traced to have originated by graded progressive stages from the simpler ones of higher Apocynaceae.” Recent cladistic analyses using morphological (Judd et al., 1994; Struwe et al., 1994) and molecular (Civeyrel, 1996; Civeyrel et al., 1998; Sennblad, 1997; Sennblad & Bremer, 1996, 2000; Sennblad et al., 1998) data have supported the contention that Asclepiadaceae and Periplocaceae are derived from within a larger, monophyletic Apocynaceae. The data presented here (Fig. 1) add further support to the naturalness of the Apocynaceae sensu lato by demonstrating that the Periplocaceae and Asclepiadaceae form monophyletic clades derived from within the higher Apocynaceae. We therefore recognize that the Apocynaceae s.l. consist of the subfamilies Periplocoideae, Secamonoideae, and Asclepiadaceae based on monophyly, but Apocynoideae and especially Rauvolfioideae are decidedly paraphyletic as delimited by Endress and Bruyns (2000). Our data therefore do not support recognition of Apocynoideae or Rauvolfioideae. Either the well-supported tribes can be maintained or many new subfamilies should be recognized (Sennblad, 1997; Civeyrel et al., 1998). We feel, however, that additional evidence is needed before such major classification changes are made. Sennblad and Bremer's (1996, 2000) phylogenetic analysis based on the *rbcL* gene suggested that Periplocaceae are most closely related to members of tribes Echiteae (*Prestonia* and *Mandevilla*) and Apocyneae (*Apocynum*). The Asclepiadaceae were, in turn, resolved as the sister group of this clade. In contrast, Civeyrel's (1996; Civeyrel et al., 1998) results, based on the *matK* locus,

showed the Periplocaceae and Asclepiadaceae to be sister taxa, with members of the Echiteae (*Prestonia*), Apocyneae (*Apocynum*), and Wrighteae (*Beaumontia*) forming a more basal clade. Both of these resolutions cannot be simultaneously correct, and, indeed, our analysis of characters from the *trnL* intron and the *trnL*-F spacer region (Fig. 1) echoes the need for further information to resolve this ambiguity.

SUBTRIBE CARISSINAE: THE SISTER GROUP TO
HIGHER APOCYNACEAE

The condition of congenital syncarpy in tribe Carisseae sensu Leeuwenberg (1994) is polyphyletic (Fig. 1). The genus *Melodinus* (subtribe Melodininae) and the genera *Hunteria*, *Picalima*, and *Pleiocarpa* (subtribe Pleiocarpinae sensu Leeuwenberg, 1994; marginally monophyletic in **78/80%** of jackknife replicates) form part of a trichotomy at two nodes less derived (Fig. 1). *Acokanthera* and *Carissa* (subtribe Carissinae sensu Leeuwenberg, 1994) form a strong group (**98/100%**) that is supported (**81/77%**) as sister to the rest of the higher Apocynaceae (including the Periplocaceae and Asclepiadaceae). This is in agreement with findings of other recent molecular studies (Sennblad & Bremer, 1996; Endress et al., 1996; Civeyrel et al., 1998). Indeed, *Acokanthera* and *Carissa* were considered congeneric by Pichon (1948). These fleshy-fruited genera with plain style-heads and non-synorganized anthers have previously been considered among the most plesiomorphic taxa of “Carisseae” and, indeed, the Apocynaceae as a whole (Endress et al., 1996; Leeuwenberg, 1994). Phylogenetic evidence from *rbcL* and *matK* data also refutes a basalmost position of the relatively unspecialized “Carisseae,” but neither gene discerns the position of subtribe Carissinae as sister to the derived clades of the Apocynaceae s.l. (Civeyrel, 1996; Sennblad & Bremer, 1996).

trnL INTRON AND *trnL*-F SPACER COMPARISON TO
RECENT PHYLOGENETIC IDEAS

The strict consensus tree from parsimony analysis of *trnL* intron and *trnL*-F spacer sequence and propagule characters for the Apocynaceae s.l. highlights discrepancies with the Endress and Bruyns (2000) hypothesis (Fig. 2). In this tree tribal delimitations in subfamily Rauvolfioideae sensu Endress and Bruyns (2000) are well supported, except for the following difference (Fig. 2, p. 533): the Alstonieae is polyphyletic—*Tonduzia* was grouped with *Alstonia* in tribe Alstonieae. In con-

trast, the genera *Tonduzia* and *Laxoplumeria* appear to be weakly associated with tribe Vinceae, which is in turn paraphyletic, with *Amsonia* grouping with *Thevetia peruviana* (Fig. 2, p. 533). Additionally, the Alyxieae appear to be polyphyletic: *Pteralyxia* (Alyxieae) is strongly associated with *Thevetia ahouai* (76/75%), which in turn is firmly nested within the Plumerieae (Fig. 2, p. 534). Note, however, that the genus *Thevetia* is polyphyletic in this phylogenetic tree; this could be interpreted as support for recognition of *Cascabela* as a genus distinct from *Thevetia*. These results require further study as the position of *T. peruviana* conforms to the expected position in studies by Sennblad and Bremer (1996) and Endress et al. (1996). Apocynoideae have very little resolution and, although weakly supported, the position of subfamily Periplocoideae appears to be nested within it (Fig. 2, p. 534). Subfamily Secamonoideae, represented by *Secamone*, is well supported (Fig. 2, p. 535), as is the Asclepiadoideae, the most highly derived subfamily (Fig. 2, p. 535). The tribe Marsdenieae is polyphyletic, with the genus *Fockea* representing the basalmost clade of the Asclepiadoideae and the *Dischidia*, *Hoya*, *Micholitzia*, *Dregea*, *Marsdenia*, *Telosma* clade sister to the Ceropegieae, the most derived tribe (Fig. 2, p. 535). Recognition of the tribe Fockeae (Kunze et al., 1994) is supported (Fig. 2, p. 535).

The remaining discussion largely follows the classification system sensu Endress and Bruyns (2000) as it is more congruent with our phylogenetic results (Fig. 2) than other current systems.

THE BASAL CLADES OF THE APOCYNACEAE

Analysis of both the molecular and the combined data matrix provides strong support for the basalmost clades of the Apocynaceae s.l. Previous molecular studies that used *rbcL* and *matK* sequence (Civeyrel, 1996; Endress et al., 1996; Sennblad & Bremer, 1996; Civeyrel et al., 1998) identified the genus *Alstonia* s. str. as the most plesiomorphic lineage, but important taxa from the Plumerieae and Alyxieae, namely *Aspidosperma* and allies, were not included in their analyses. The results of this study show that a clade comprising *Aspidosperma*, *Geissospermum*, *Microplumeria*, *Haplophyton*, *Strepeliopsis*, and *Vallesia* is sister to the rest of the family (Fig. 2, p. 533; both the clade and its basal position are supported by 100/100% and 94/96% of jackknife replicates, respectively). These genera belong to the Alstonieae (Fig. 2, p. 533). In keeping with earlier molecular results, the next most basal taxon after this

clade is *Alstonia* s. str. (Alstonieae; supported by 95/97% of jackknife replicates). The genus *Tonduzia*, which has been synonymized with *Alstonia* (Pichon, 1947a; Gentry, 1983) and maintained in the latest monograph in *Alstonia* (Sidiyasa, 1998) was recognized by Endress and Bruyns (2000), who, however, placed both *Alstonia* and *Tonduzia*, as well as the poorly known genus *Laxoplumeria*, in tribe Alstonieae. In contrast, in the present phylogenetic study, there is no support for a close relationship between *Alstonia* and the latter two genera. Rather, both *Tonduzia* and *Laxoplumeria*—both from tropical America—appear to be weakly associated with tribe Vinceae, i.e., in a more derived position than the African and Asian *Alstonia* (Fig. 2). As noted above, the group traditionally held as most plesiomorphic, the Carisseae, is syncarpous, but is also highly derived (Fig. 2, p. 534). Members of tribes Carisseae and Alstonieae share relatively unspecialized flowers that have a uniformly receptive and secretory style-head and non-synorganized stamens. Species of *Haplophyton* and *Vallesia* have a vertically differentiated style-head, with basal stigmatic regions (characteristic of most Apocynaceae s.l.). Moreover, *Haplophyton* has stamens that are convergent over the style-head as do more derived members of the family, e.g., *Allamanda*, *Rauwolfia*, and most species of *Tabernaemontana* (Endress, 1986; Endress et al., 1996; Leeuwenberg, 1994; Pichon, 1950b; Potgieter, unpublished). *Alstonia* s. str., on the other hand, displays a vertically differentiated style-head without convergent stamens (Endress et al., 1996). Therefore, style-head specialization and the beginnings of synorganization appear to have evolved in parallel among lower clades of the Apocynaceae s.l., becoming fixed among the higher clades, but with a notable reversal in the tribe Carisseae. The molecular-derived relationships of the Apocynaceae s.l. to outgroup taxa remain unclear using both *trnL* intron and *trnL-F* spacer sequences (Struwe et al., 1998; pers. comm.) as well as data from the *matK* and *rbcL* regions (Civeyrel, 1996; Civeyrel et al., 1998; Endress et al., 1996; Sennblad & Bremer, 1996). The Loganiaceae genera *Gardneria*, *Strychnos*, and *Usteria* appear to be morphologically similar in that they have flattened seeds with a central hilum (Leeuwenberg & Leenhouts, 1980; Struwe et al., 1994) in common with the genus *Aspidosperma* (Alstonieae).

EVOLUTION OF PROPAGULE CHARACTERS

Most examinations of character evolution in the Apocynaceae s.l. have centered on issues related

to floral evolution, i.e., synorganization and specialization of the androecial and gynoecial structures (e.g., Endress et al., 1996; Endress, 1994; Fallen, 1986; Judd et al., 1994; Kunze, 1990, 1993, 1996; Nilsson et al., 1993; Safwat, 1962). While floral complexity does increase with increasing phylogenetic derivation (Civeyrel, 1996; Endress et al., 1996; Sennblad & Bremer, 1996), associated traits (related to synorganization and specialization) are by no means fixed until the uppermost branches of the Apocynoideae (i.e., above the Carisseae) are reached. This is readily apparent when floral characters are optimized onto our molecular trees (unpublished results) and is clear from our discussion here of parallel trends of synorganization and specialization in the basalmost clades of Apocynaceae s.l. Indeed, with members of the Apocynaceae s.l. bearing the most complex flowers of any dicotyledonous angiosperm (Endress et al., 1983; Endress, 1994), to do full justice to the issue of floral character evolution will require many additional ontogenetic studies beyond those that have so far been performed.

Instead of focusing on these most popularly studied attributes of the Apocynaceae s.l., a subject that has not heretofore received such attention, the phylogenetic correlates of propagule characters, is presented. Several fruit and seed characteristics, likely correlated with dispersal mode, have been used in traditional classifications, but with the exception of syncarpy (Endress et al., 1996; Sennblad & Bremer, 1996), their phylogenetic and evolutionary significance has not yet been explored.

Most members of the Gentianales have syncarpous ovaries, but apocarpy is common among Apocynaceae s.l. and is the rule for Loganiaceae genera *Mitrasacme* and *Mitreola*, which are apically apocarpous (Conn & Brown, 1996; Endress et al., 1983; Fallen, 1983b, 1986; Struwe et al., 1994). Analysis of data from plastid genes, *matK*, *rbcL*, and *trnL-F* (Civeyrel, 1996; Sennblad & Bremer, 1996; Civeyrel et al., 1998; Sennblad, 1997; Sennblad & Bremer, 2000; this paper, Fig. 2), indicates that apocarpy is the plesiomorphic state for the Apocynaceae s.l. (see above and Endress et al., 1996). In the Gentianaceae at least, congenital syncarpy is thought to be a prerequisite for the development of fleshy fruits (Struwe, 1999). In the Apocynaceae s.l., on the other hand, fleshy fruits are known among both congenitally syncarpous as well as apocarpous taxa. Fleshy fruits are often associated with animal-mediated dispersal syndromes, and are evaluated within a phylogenetic context.

Mesocarp is the differentiated ovary wall, excluding the inner and outer layers (endocarp and exocarp, respectively). Dry mesocarp is plesiomorphic for the Apocynaceae s.l., whereas fleshiness has been derived independently multiple times within this clade (Table 2). Fleshy mesocarp is known from the early-branching *Vallesia* and *Geissospermum* (Alstonieae), the relatively early-branching, syncarpous Willughbeeae (*Couma*, *Paccouria*, *Saba*, *Lacmellea*, and *Landolphia*), the Tabernaemontaneae clade (most of which are apocarpous except for members of the former Ambelanieae), *Rauwolfia* (including both syncarpous and apocarpous species), *Neisosperma* and *Ochrosia* (Vinceae), the more derived and syncarpous genus *Melodinus* of the Melodineae, the apocarpous Hunterieae (*Hunteria*, *Picralima*, and *Pleiocarpa*), some syncarpous (*Lepiniopsis*) as well as apocarpous (*Alyxia*, *Pteralyxia*) Alyxieae, and *Cerbera* and *Thevetia* (Plumerieae), the syncarpous Carisseae (*Acokanthera* and *Carissa*) and *Thevetia ahouai*, which in this analysis, shows a weak relationship with *Amsonia* (Vinceae). Fleshy mesocarp, which has been used as a taxonomic character to unify Carisseae in traditional classifications (e.g., Leeuwenberg, 1994), is clearly inconsistent with the phylogenetic placement of the various syncarpous clades assigned to that tribe. Fleshiness probably correlates more with ecological traits, e.g., dispersal syndrome, than with anything else.

Endocarp in the Apocynaceae is primitively non-sclerified (Table 2). Sclerification of the endocarp is often associated with drupe development. Sclerified endocarp appears to have been derived in the Apocynaceae s.l. six times independently (Table 2), sometimes, though not always, in correlation with fleshy mesocarp. Of the basal genera, *Strempeleopsis*, *Haplophyton*, *Microplumeria*, *Aspidosperma*, and *Alstonia* have dry fruit. *Vallesia* has a fleshy drupe, and *Geissospermum* has a fleshy fruit, best described as a berry; and both *Neisosperma* and *Ochrosia* have a fleshy mesocarp. The dry, stringy layers in some genera, e.g., *Neisosperma*, are outgrowths of the endocarp; the mesocarp, however, is fleshy and rots away relatively quickly, leaving the thick endocarp outgrowths that form a thick, dry covering that serves for long-distance dispersal. The endocarp of *Aspidosperma* and *Vallesia* (Alstonieae sensu Endress & Bruyns, 2000) and *Neisosperma* and *Ochrosia* (Vinceae sensu Endress & Bruyns, 2000) is sclerified. Syncarpous *Lepiniopsis* and hemi-syncarpous *Rauwolfia serpentina* as well as the apocarpous Alyxieae and Plumerieae (sensu Endress

& Bruyns, 2000) have fleshy drupes with a sclerified endocarp; *Skytanthus* and *Cerberiopsis* have dry and fibrous mericarps, and *Alyxia* has fleshy drupe-like articles, which are detached, single-seeded portions of moniliform follicles (Middleton, 2000). Notably, taxa of the fleshy-fruited Carisseae have a non-sclerified endocarp. Sclerification of the endocarp is a specialization not well correlated with the bearing of fleshy drupes, as some sclerified taxa are certainly not drupaceous (e.g., *Aspidosperma*).

Seed presentation in the Apocynaceae s.l. is primitively naked, i.e., without a distinct aril and not embedded in a well-developed pulp (Table 2). The transition from naked seeds to seeds embedded in pulp appears to have occurred several times unambiguously within the Apocynaceae s.l., whereas the transformation from arillate to embedded-in-pulp has only occurred three times independently. The transition from naked to arillate is never unambiguous on the molecular-only tree (not shown), but very likely occurred in the ancestor of the Tabernaemontaneae clade. Independent origins of fruit pulp have occurred in *Geissospermum* (Alstonieae), *Couma* through *Saba* (Willughbeeae), *Molongum* (Tabernaemontaneae), *Melodinus* (Melodineae), Hunterieae, and the Carisseae. The arillate and pulp-embedded states are partly correlated with mesocarp fleshiness, conforming to the syndrome of features favoring animal-mediated seed dispersal.

The seed margin is primitively winged to naked in the Apocynaceae s.l. (Table 2). Wing homology is difficult to establish in phylogenetically basal and derived taxa. The question whether seed wings of the genus *Aspidosperma* (basal) are homologous to those found in the subfamilies Secamonoideae and Asclepiadoideae remains undecided, but these features were treated as homologous in this study. Independent origins of seed wings have occurred multiple times in the subfamilies Rauvolfioideae, Periplocoideae, and the Asclepiadoideae. Members of the genus *Aspidosperma* (Alstonieae) have the largest winged seed of the Apocynaceae s.l., and include both non-circumalate and circumalate seed. The wings of *Plumeria* seeds (Plumerieae) are non-circumalate, while those of *Strepeliopsis* (Alstonieae), *Tonduzia* [*Alstonia* (see Leeuwenberg, 1994) for a differing opinion], *Plectaneia*, and *Cerberiopsis*, although subject to different interpretation, were treated as having incompletely circumalate winged seeds. Of the members of the Periplocoideae, members of the genus *Mondia* have narrow seed wings (pers. obs.; see Swarupanandan et al., 1996, for a dif-

ferent opinion and for further discussion). As a rule, the seeds of members of the Asclepiadoideae have a very narrow flat margin that has sometimes been called winged, but homology to the large, well-defined wings in other parts of the family is uncertain. This character has been secondarily lost in a number of species in the following genera: *Mateleia*, *Oxystelma*, *Gomphocarpus*, and *Hoya* (Stevens, pers. comm.).

The seed coma is primitively absent in the Apocynaceae s.l. (Table 2). The character state "ill-defined comas" refers to seed margins that surround the entire seed margin, are of uniform length, and are ciliate. Ill-defined comas have been defined as wings, but without thorough ontogenetic investigation these classifications are subject to interpretation; in this context, *Alstonia*, *Tonduzia*, and *Laxoplumeria* have ill-defined comas. Well-defined seed comas have localized areas that are highly pubescent; this character is present in the Apocynoideae, Periplocoideae, Secamonoideae, and the Asclepiadoideae. A well-defined seed coma appears to have been derived from an immediate ancestor with the syncarpous, fleshy-fruited members of the Carisseae (*Carissa* and *Acokanthera*), although this relationship is only moderately supported by a jackknife value of 81/77% (Fig. 2, p. 534). The potential transition from a syncarpous fruit with seeds embedded in fleshy pulp to an apocarpous fruit with greatly increased numbers of small seeds with both wings and a well-defined coma was unexpected. The fleshy-fruited condition is most likely autapomorphic for the Carisseae. Given that most of the family has seeds with well-defined comas, the evolution of this character can be used to hypothesize the derivation of four of five extant subfamilies via radiative expansion.

The production of small seeds with a coma may have been the evolutionary step necessary for cladogenesis/speciation through increased dispersal and exposure to new habitats. As a likely result of coma-mediated seed dispersal, the Apocynaceae s.l. are found worldwide.

Geography in the Apocynaceae s.l. appears to be primitively Gondwanan (South America, Africa, and the Indian Ocean Basin) from non-additive optimization of area states (see Ross & Scotese, 1988, and Unternehr et al., 1988, for discussion on tectonic models; Ronquist, 1994; see Albert & Struwe, 1997, for an example of area optimization). This is useful information for estimating the age of origination for the genus *Aspidosperma* and its close neotropical associates, which must then have had a western Gondwanaland distribution.

Future research could be directed at integrated morphological and molecular-developmental studies of both pollination and seed dispersal traits to better understand the origins and mechanistic extents of both parallel and innovative reproductive evolution in this large angiosperm family.

Literature Cited

*An asterisk indicates a primary literature source for fruit and seed characters related to dispersal mode (see Table 2).

- Albert, V. A. & L. Struwe. 1997. Phylogeny and classification of *Voyria* (saprophytic Gentianaceae). *Brittonia* 49: 466–479.
- Allorge, L. 1980.* *Considérations sur la systématique des Apocynacées*. Pp. 112–126 in L. Allorge, H. P. Husson & C. Sastre (editors), *Morphologie et chimio-taxonomie des Apocynacées: Conclusions phylogénétiques et biogéographiques*. *Compt. Rend. Séances Soc. Biogéogr.* 57.
- . 1985a.* *Contribution à l'étude des graines des Apocynaceae–Tabernaemontanoideae: Origine de l'arille et ornementation du tegument séminal*. *Adansonia* 7: 433–451.
- . 1985b.* *Monographie des Apocynacées–Tabernaemontanoïdées Américaines. Morphologie, Systématique, Chimio-taxonomie*. *Mém. Mus. Natl. Hist. Nat., Sér. B, Bot.* 30: 1–119.
- Beentje, H. J. 1982.* *A monograph on Strophanthus DC. (Apocynaceae)*. *Meded. Landbouwhogeschool* 83: 21–52.
- Bergen, M. van & W. Snoeijer. 1996.* *Catharanthus*. The Madagascar periwinkle and related species. *Wageningen Agric. Univ. Pap.* 96(3): 1–120.
- Boom, R., C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim van Dillen & J. van der Noordaa. 1990. Rapid and simple methods for purification of nucleic acids. *J. Clin. Microbiol.* 28: 495–503.
- Brown, N. E. 1904.* *Asclepiadeae*. Pp. 231–503 in W. T. Thiselton-Dyer (editor), *Flora of Tropical Africa* 4(1). L. Reeve, London.
- . 1909.* *Asclepiadeae*. Pp. 518–1036 in W. T. Thiselton-Dyer (editor), *Flora Capensis* 4(1). L. Reeve, London.
- Brown, R. 1810. On the Asclepiadeae, a natural order of plants separated from the Apocineae of Jussieu. [Preprint of *Mem. Wern. Nat. Hist. Soc.* 1: 12–78 (1811).]
- Chávez de Michel, L. R. 1993.* *Apocynaceae*. Pp. 114–126 in T. J. Killeen, E. García E. & S. G. Beck (editors), *Guía de Arboles de Bolivia*. Editorial del Instituto de Ecología, La Paz, Bolivia.
- Civeyrel, L. 1996. *Phylogénie des Asclepiadaceae, approche Palynologique et Moléculaire*. Ph.D. Dissertation, Montpellier.
- , A. le Thomas, K. Ferguson & M. W. Chase. 1998. Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to the molecular phylogeny obtained from plastid *matK* sequences. *Molec. Phylogenet. Evol.* 9: 517–527.
- Conn, B. J. & E. A. Brown. 1996.* *Strychnos*. Pp. 64–66 in A. E. Orchard & A. Wilson (editors), *Flora of Australia*, Vol. 28. Gentianales. CSIRO Australia, Melbourne.
- Degener, O. 1946.* *Apocynaceae and Asclepiadaceae. Families 305 & 306 (no pages) in O. Degener (editor), Flora Hawaiiensis; The New Illustrated Flora of the Hawaiian Islands 1–4*, Honolulu.
- Demeter, K. 1922. *Vergleichende Asclepiadenstudien*. *Flora* 115: 130–176.
- Dilst, F. J. H. van. 1995.* *Baisseia* A. DC. Series of revisions of Apocynaceae XXXIX. *Bull. Jard. Bot. Belg.* 64: 89–178.
- Doyle, J. J. & J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11–15.
- Endress, M. E. 1995.* *Condylocarpon*. Pp. 492–493 in J. A. Steyermark, P. E. Berry & B. K. Holst (editors), *Flora of the Venezuelan Guayana*, Vol. 2. Timber Press, Portland, and Missouri Botanical Garden Press, St. Louis.
- & P. V. Bruyns. 2000. A revised classification of Apocynaceae s.l. *Bot. Rev. (Lancaster)* 66: 1–56.
- , M. Hesse, S. Nilsson, A. Guggisberg & J. Zhu. 1990.* The systematic position of the Holarrheninae (Apocynaceae). *Pl. Syst. Evol.* 171: 157–185.
- , B. Sennblad, S. Nilsson, L. Civeyrel, M. W. Chase, S. Huysmans, E. Grafström & B. Bremer. 1996.* A phylogenetic analysis of the Apocynaceae s. str. and some related taxa in Gentianales: A multidisciplinary approach. *Opera Bot. Belg.* 7: 59–102.
- Endress, P. K. 1994. *Gentianales (Asteridae). Asclepiadaceae*. Pp. 302–319 in *Diversity and Evolutionary Biology of Tropical Flowers*. Cambridge Univ. Press, Cambridge.
- , M. Jenny & M. E. Endress. 1983. Convergent elaboration of apocarpous gynoecea in higher advanced dicotyledons (Sapindales, Malvales, Gentiales). *Nordic J. Bot.* 3: 293–300.
- Ezcurra, C. 1981.* *Revisión de las Apocinaceas de la Argentina*. *Darwiniana* 23: 367–474.
- , M. E. Endress & A. J. M. Leeuwenberg. 1992.* *Apocynaceae*. Pp. 1–120 in R. Spichiger & L. Ramella (editors), *Flora del Paraguay* 17. Conservatoire et Jardin Botaniques de la Ville de Genève and Missouri Botanical Garden, St. Louis.
- Fallen, M. E. 1983a.* *A taxonomic revision of Condylocarpon (Apocynaceae)*. *Ann. Missouri Bot. Gard.* 70: 149–169.
- . 1983b. *Morphological, Functional, and Evolutionary Aspects of the Flower in the Apocynaceae*. Ph.D. Dissertation, Zurich.
- . 1985.* *The gynoeccial development and systematic position of Allamanda (Apocynaceae)*. *Amer. J. Bot.* 72: 572–579.
- . 1986. *Floral structure in the Apocynaceae: Morphological, functional, and evolutionary aspects*. *Bot. Jahrb. Syst.* 106: 245–286.
- Farris, J. S. 1989. The retention index and homoplasy excess. *Syst. Zool.* 38: 406–407.
- , V. A. Albert, M. Källersjö, D. Lipscomb & A. G. Kluge. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124.
- Fitch, W. M. 1971. Toward defining the course of evolution: Minimum change for a specific tree topology. *Syst. Zool.* 20: 406–416.
- Forster, P. I. & D. J. Liddle. 1996.* *Hoya, Dischidia*. Pp. 197–283 in A. E. Orchard & A. Wilson (editors), *Flora of Australia*, Vol. 28. Gentianales. CSIRO Australia, Melbourne.

- Gentry, A. H. 1974.* Notes on Panamanian Apocynaceae. *Ann. Missouri Bot. Gard.* 61: 891–900.
- . 1983. *Alstonia* (Apocynaceae): Another paleotropical genus in Central America. *Ann. Missouri Bot. Gard.* 70: 206–207.
- Hansen, B. F. 1995.* *Forsteronia*. Pp. 496–499 in J. A. Steyermark, P. E. Berry & B. K. Holst (editors), *Flora of the Venezuelan Guayana*, Vol. 2. Timber Press, Portland, & Missouri Botanical Garden Press, St. Louis.
- Hooker, J. D. 1882.* Apocynaceae. Pp. 621–672 in J. D. Hooker (editor), *The Flora of British India* 3(1). L. Reeve, London.
- Hutchinson, J. & J. M. Dalziel. 1931.* Apocynaceae and Asclepiadaceae. Pp. 28–65 in J. Hutchinson & J. M. Dalziel (editors), *Flora of West Tropical Africa* 2. The Crown Agents for the Colonies, London.
- Jarvie, J. K. & Ermayanti. 1997.* *Cerbera* in Tree Genera of Borneo—Descriptions and Illustrations [current web address: www.huh.harvard.edu/databases/legacy/navi-key/phillipsia.html].
- Jepson, W. L. 1923.* Apocynaceae and Asclepiadaceae. Pp. 768–775 in W. L. Jepson (editor), *A Manual of the Flowering Plants of California*. Univ. California Press, Berkeley.
- Jhingan, A. K. 1992. A novel technology for DNA isolation. *Meth. Molec. Biol.* 3: 15–22.
- Judd, W. S., R. W. Sanders & M. J. Donoghue. 1994. Angiosperm family pairs: Preliminary phylogenetic analyses. *Harvard Pap. Bot.* 1(5): 1–51.
- Jussieu, A. L. de. 1789. *Genera Plantarum*. Herissant, Paris.
- Kunze, H. 1993. Evolution of the translator in Periplocaceae and Asclepiadaceae. *Pl. Syst. Evol.* 185: 99–122.
- . 1990. Morphology and evolution of the corona in Asclepiadaceae and related families. *Trop. Subtrop. Pflanzenwelt* 76: 1–51.
- . 1996. Morphology of the stamen in the Asclepiadaceae and its systematic relevance. *Bot. Jahrb. Syst.* 118: 547–579.
- , U. Meve & S. Liede. 1994. *Cibirhiza albersiana*, a new species of Asclepiadaceae, and establishment of the tribe Fockeeae. *Taxon* 43: 367–376.
- Kupicha, F. K. 1981.* Studies on African Apocynaceae: The genus *Acokanthera*. *Kew Bull.* 37: 40–67.
- Leeuwenberg, A. J. M. 1983.* Some remarks on the taxonomy of the Plumerioideae (Apocynaceae). *Bothalia* 14: 799–801.
- . 1991.* A Revision of *Tabernaemontana*: The Old World Species. Royal Botanic Gardens Kew/Whitstable Litho, Whitstable.
- . 1994.* Taxa of the Apocynaceae above the genus level. Series of Apocynaceae XXXIII. Wageningen Agric. Univ. Pap. 94(3): 47–60.
- . 1995.* *Tabernaemontana*. Pp. 562–569 in J. A. Steyermark, P. E. Berry & B. K. Holst (editors), *Flora of the Venezuelan Guayana*, Vol. 2. Timber Press, Portland, & Missouri Botanical Garden Press, St. Louis.
- & P. W. Leenhouts. 1980. Taxonomy. Pp. 8–96 in A. J. M. Leeuwenberg (editor), A. Engler & K. Prantl's *Die Natürlichen Pflanzenfamilien, Angiospermae: Ordnung Gentianales, Fam. Loganiaceae*, Vol. 28b. Duncker & Humblot, Berlin.
- Liede, S. 1997.* Subtribes and genera of the tribe Asclepiadeae (Apocynaceae, Asclepiadoideae)—A synopsis. *Taxon* 46: 233–247.
- Luckow, M. & A. Bruneau. 1997. Circularity and independence in phylogenetic tests of ecological hypothesis. *Cladistics* 13: 145–151.
- Mabberley, D. J. 1997. *The Plant Book: A Portable Dictionary of the Vascular Plants*, 2nd ed. Cambridge Univ. Press, Cambridge.
- MacFarlane, J. M. 1933.* The Apocynaceae and Asclepiadaceae. Pp. 1–181 in *The Evolution and Distribution of Flowering Plants* 1. Noel Printing, Philadelphia.
- Maddison, W. P. & D. R. Maddison. 1992. *MacClade: Analysis of phylogeny and character evolution*. Ver 3.0. Computer program and documentation. Sinauer, Sunderland, Massachusetts.
- Matthew, K. M. 1995.* Apocynaceae, Asclepiadaceae, Loganiaceae. Pp. 289–311 in K. M. Matthew (editor), *An Excursion Flora of Central Tamilnadu, India*. Balkema, Rotterdam.
- Middleton, D. J. 1999.* Apocynaceae. In *Flora of Thailand*. Vol. 7(1): 1–153. The Forest Herbarium, Bangkok.
- . 2000. Revision of *Alyxia* (Apocynaceae). Part 1: Asia and Malesia. *Blumea* 45: 1–146.
- Monachino, J. 1949.* A revision of the genus *Alstonia* (Apocynaceae). *Pacific Sci.* 3: 133–182.
- Morillo, G. N. 1995.* *Catharanthus*, P. 491; *Galactophora*, Pp. 498–503; *Mandevilla*, Pp. 518–528; *Prestonia*, Pp. 548–550; *Rauwolfia*, Pp. 550–553; *Thevetia*, Pp. 570–571 in J. A. Steyermark, P. E. Berry & B. K. Holst (editors), *Flora of the Venezuelan Guayana*, Vol. 2. Timber Press, Portland, & Missouri Botanical Garden Press, St. Louis.
- Ngan, P. T. 1965.* A revision of the genus *Wrightia* (Apocynaceae). *Ann. Missouri Bot. Gard.* 52: 114–175.
- Nilsson, S., M. E. Endress & E. Grafstrom. 1993. On the relationship of the Apocynaceae and Periplocaceae. *Grana, Suppl.* 2: 3–20.
- Nowicke, J. W. 1970.* Apocynaceae. In R. E. Woodson & R. W. Schery (editors), *Flora of Panama*. *Ann. Missouri Bot. Gard.* 57: 59–130.
- Persoon, J. G. M., F. J. H. van Dilst, R. P. Kuijpers, A. J. M. Leeuwenberg & G. J. A. Vonk. 1992.* The African species of *Landolphia* P. Beauv. Series of revisions of Apocynaceae XXXIV. Wageningen Agric. Univ. Pap. 92(2): 1–232.
- Pichon, M. 1947a. Classification des Apocynacées: IV. Genre *Alstonia* et genres voisins. *Mém. Mus. Natl. Hist. Nat., sér. 2*, 19: 294–301.
- . 1947b. Classification des Apocynacées: VIII. Les *Voacanga* d'Afrique. *Mém. Mus. Natl. Hist. Nat., sér. 2*, 19: 409–416.
- . 1948.* Classification des Apocynacées. I. Carissées et Ambelaniées. *Mém. Mus. Natl. Hist. Nat., sér. 2*, 24: 111–181.
- . 1949a.* Classification des Apocynacées: XXVI. Détermination des échantillons fleuris de Plumerioïdées. *Mém. Mus. Natl. Hist. Nat., sér. 2*, 21: 140–146.
- . 1949b.* Classification des Apocynacées: XXVII. Détermination des graines de Plumerioïdées et de Cerberoidées. *Mém. Mus. Natl. Hist. Nat., sér. 2*, 21: 266–269.
- . 1950a.* Classification des Apocynacées. XXV. Echitoïdées. *Mém. Mus. Natl. Hist. Nat. Sér. B*, 1: 1–174.
- . 1950b.* Classification des Apocynacées: XXV. Echitoïdées et Supplément aux Pluméroïdées. *Mém. Mus. Natl. Hist. Nat., Sér. B*, 1: 1–174.

- Ploeg, J. van der. 1983.* A Revision of *Isonema* R. Br. and *Pycnobotrya* Benth. (Apocynaceae). Wageningen Agric. Univ. Pap. 83(4): 1–20.
- Potgieter, K. 1999. Phylogenetic Study of Apocynaceae Juss. and *Aspidosperma* Mart. & Zucc. Ph.D. Dissertation, University of Illinois, Urbana.
- & V. A. Albert. 1997. Preliminary phylogenetic study of the genus *Aspidosperma* (Apocynaceae) and closely related genera using ITS and *trnL-F* sequence data. *Amer. J. Bot.* 84 (6, Abstracts): 223.
- & ———. 1998a. *Aspidosperma* evolution based on cladistic analyses of morphological and molecular (ITS, *trnL-F*) sequence data. *Amer. J. Bot.* 85 (6, Abstracts): 151.
- & ———. 1998b. Non-floral morphological correlates of the Apocynaceae s.s.–Periplocaceae–Asclepiadaceae transition (i.e., dispersal traits) mapped onto a *trnL-F* derived consensus tree. *Amer. J. Bot.* 85 (6, Abstracts): 152.
- Ronquist, F. 1994. Ancestral areas and parsimony. *Syst. Biol.* 43: 267–274.
- Rosatti, T. J. 1989.* The genera of suborder Apocynineae (Apocynaceae and Asclepiadaceae) in the Southeastern United States. *J. Arnold Arbor.* 70: 307–401; 71: 443–514.
- Ross, M. I. & C. R. Scotese. 1988. A hierarchical tectonic model of the Gulf of Mexico and Caribbean region. *Tectonophysics* 155: 139–168.
- Rudijiman. 1986.* A revision of *Beaumontia* Wallich, *Kibatalia* G. Don and *Vallariopsis* Woodson (Apocynaceae). Wageningen Agric. Univ. Pap. 86(5): 3–44.
- Safwat, F. 1962. The floral morphology of *Secamone* and the evolution of the pollinating apparatus in Asclepiadaceae. *Ann. Missouri Bot. Gard.* 49: 95–129.
- Sanderson, M. J. & J. J. Doyle. 1993. Phylogenetic relationships in North American *Astragalus* (Fabaceae) based on chloroplast DNA restriction site variation. *Syst. Bot.* 18: 395–408.
- Savolainen, V., P. Cuénoud, R. Spichiger, M. D. P. Martinez, M. Crèvecoeur & J. F. Manen. 1995. The use of herbarium specimens in DNA phylogenetics: Evaluation and improvement. *Pl. Syst. Evol.* 197: 87–98.
- Schlechter, F. R. R. 1905. Periplocaceae. Pp. 351–353 in K. Schumann & K. Lauterbach (editors), *Nachträge zur Flora des Deutschen Schutzgebiete in der Sudsee*. Borntraeger, Leipzig.
- Sennblad, B. 1997.* Is there a justification for differential *a priori* weighting in coding sequences?—A case study from *rbcL* and Apocynaceae. Ph.D. Dissertation, Acta Universitatis Upsaliensis, Uppsala.
- & B. Bremer. 1996. The familial and subfamilial relationships of Apocynaceae and Asclepiadaceae evaluated with *rbcL* data. *Pl. Syst. Evol.* 202: 153–175.
- & ———. 2000. Is there a justification for differential *a priori* weighting in coding sequence?—A case study from *rbcL* and Apocynaceae. *Syst. Biol.* 49: 43–55.
- , M. E. Endress & B. Bremer. 1998. Morphology and molecular data in phylogenetic fraternity—The tribe Wrightieae (Apocynaceae) revisited. *Amer. J. Bot.* 85: 1143–1158.
- Sidiyasa, K. 1998. Taxonomy, phylogeny and wood anatomy of *Alstonia* (Apocynaceae). *Blumea Suppl.* 11: 1–230.
- Small, J. K. 1903.* Apocynaceae and Asclepiadaceae. Pp. 933–956 in J. K. Small (editor), *Flora of the South-eastern United States*. [Published by the author] New York.
- . 1933.* Apocynaceae and Asclepiadaceae. Pp. 1057–1078 in J. K. Small (editor), *Manual of the South-eastern Flora*. New York.
- Smith, A. C. 1988.* Apocynaceae and Asclepiadaceae. Pp. 43–123 in A. C. Smith (editor), *Flora Viteinsis Nova. A New Flora of Fiji 4*. Pacific Tropical Botanical Garden, Hawaii.
- Souza, L. A. D. & I. S. Moscheta. 1992.* Morfo-anatomia do fruto e da plântula de *Aspidosperma polyneuron* M. Arg. (Apocynaceae). *Revista Brasil. Biol.* 52: 439–447.
- Stapf, O. 1904.* Apocynaceae. Pp. 24–233 in W. T. Thistelton-Dyer (editor), *Flora of Tropical Africa 4*. L. Reeve, London.
- Stevens, W. D. 1975. Notes on the genus *Matelea* (Apocynaceae, s.l.). *Phytologia* 32: 387–406.
- . 1988.* New names and combinations in Apocynaceae, Asclepiadoideae. *Phytologia* 64: 333–335.
- Struwe, L. 1999. Morphological and Molecular Phylogenetic Studies in Neotropical Gentianaceae. Ph.D. Dissertation, Stockholm University, Sweden.
- & V. A. Albert. 1997. Floristics, cladistics, and classification: Three case studies in Gentianales. Pp. 321–352 in J. Dransfield, M. J. E. Coode & D. A. Simpson (editors), *Plant Diversity in Malesia III*. Royal Botanical Gardens, Kew.
- , ——— & B. Bremer. 1994 [1995]. Cladistics and family level classification of the Gentianales. *Cladistics* 10: 175–206.
- , M. Thiv, J. W. Kadereit, A. S. R. Pepper, T. J. Motley, P. J. White, J. H. E. Rova, K. Potgieter & V. A. Albert. 1998. *Saccifolium* (Saccifoliaceae), an endemic of Sierra de la Neblina on the Brazilian-Venezuelan frontier, is related to a temperate-alpine lineage of Gentianaceae. *Harvard Pap. Bot.* 3(2): 199–214.
- Swarupandan, K., J. K. Mangaly, T. K. Sonny, K. Kishorekumar & S. Chand Basha. 1996.* The subfamilial and tribal classification of the family Asclepiadaceae. *Bot. J. Linn. Soc.* 120: 327–369.
- Swofford, D. L. 1998. PAUP-Phylogenetic Analysis Using Parsimony (and Other Methods), version 4.0.0d64. Computer program distributed by Swofford & Sinauer, Sunderland, Massachusetts.
- Taberlet, P., L. Gielly, G. Patou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.
- Thorne, R. F. 1992. Classification and geography of the flowering plants. *Bot. Rev.* 58: 225–348.
- Unternehm, P., D. Curie, J. L. Olivet, J. Goslin & P. Beuzart. 1988. South Atlantic fits and intraplate boundaries in Africa and South America. *Tectonophysics* 155: 169–179.
- Vonk, G. J. A. & A. J. M. Leeuwenberg. 1989.* A taxonomic revision of the genus *Tabernaemontana* and a study of wood anatomy of *T. iboga*. Wageningen Agric. Univ. Pap. 89(4): 3–18.
- Williams, J. B. 1996.* *Mandevilla* and *Parsonsia*. Pp. 104–196 in A. E. Orchard & A. Wilson (editors), *Flora of Australia*, Vol. 28. Gentianales. CSIRO Australia, Melbourne.
- Woodson, R. E., Jr. 1935.* Studies in the Apocynaceae IV. The American genera of Echitoideae V. *Forsteronia*, etc. *Ann. Missouri Bot. Gard.* 22: 153–306.

-
- . 1936.* Studies in the Apocynaceae IV. The American genera of Echioideae. *Ann. Missouri Bot. Gard.* 23: 169–438.
- . 1938.* Apocynaceae. *N. Amer. Flora* 29: 103–192.
- Zarucchi, J. L. 1987.* Revision of the Tribe Ambelanieae (Apocynaceae–Plumerioideae). *Wageningen Agric. Univ. Pap.* 87(1): 22–89.
- . 1995.* *Couma*, Pp. 494–495; *Himatanthus*, Pp. 505–508; *Lacmellea*, Pp. 509–511; *Macoubea*, Pp. 511–512; *Microplumeria*, Pp. 531–532; *Molongum*, Pp. 532–533; *Pacouria*, Pp. 542–543; *Plumeria*, Pp. 546–547 in J. A. Steyermark, P. E. Berry & B. K. Holst (editors), *Flora of the Venezuelan Guayana Vol. 2*. Timber Press, Portland, & Missouri Botanical Garden Press, St. Louis.

Appendix 1. List of accessions sampled for DNA used for the Apocynaceae s.l. plastic *trnL* intron and *trnL-F* spacer study. Subfamilial and tribal designations are provided in the first two columns. Sample identification, place of collection (where known), and voucher information are given in the next three columns. GenBank accession numbers for deposited *trnL* intron and *trnL-F* spacer sequences are given in the last two columns, respectively. Specimen voucher information includes collector and collection number, location of voucher and/or living material. Herbarium acronyms follow Index Herbariorum: AAU, BR, C, CONN, DAV, FTG, GA, ILL, MO, NOU, NY, WAG, Z (* = permanent anatomical slides of flowers; M. Endress).

Loganiaceae				GenBank Sequence		
Outgroups	Country	Voucher		<i>trnL</i>	<i>trnL-F</i>	
	Venezuela, Bolivar	Berry & Brako 5525 (NY)		AF102386	AF214146	
	French Guiana, Région de Saül	Mori 24166 (NY)		AF102484	AF214147	
				GenBank Sequence		
Subfamily	Tribe	Apocynaceae s.l.	Country	Voucher		
Apocynoideae	Apocynae	<i>Apocynum androsaemifolium</i> L.	U.S.A.	Potgieter & Cahilly 238 (NY)	AF214308	AF214154
		<i>Apocynum cannabinum</i> L.	U.S.A.	Potgieter & Cahilly 239 (NY)	AF102380	AF214155
		<i>Baissea leonensis</i> Benth.	Cameroun, Etonde- Fang	DeKruif 1067 (WAG) 81-0058 (BR) 890007 (CONN)	AF214320	AF214166
		<i>Beaumontia grandiflora</i> Wall.		81-0058 (BR)	AF214321	AF214167
		<i>Chonemorpha fragrans</i> (Moon) Alston		Forster 20091 (Z*)	AF214334	AF214180
				941310 (FTG)	AF214335	AF214181
				07-3805, Billiet 3530 (BR)	AF214336	AF214182
		<i>Forsteronia corymbosa</i> (Jacq.) G. Mey.	Puerto Rico	Struwe 1093 (NY)	AF214355	AF214201
		<i>Trachelospermum jasminoides</i> Lem.		851419 (CONN)	AF214439	AF214285
		<i>Urceola rosea</i> (Hook. & Arn.) D. J. Middleton		Forster 20090 (Z*)	AF214445	AF214291
		<i>Cycladenia humilis</i> Benth.	U.S.A., California	Smith & Rultan 520 (Z)	AF214341	AF214187
		<i>Echites umbellata</i> Jacq.		Correll 45549 (NY)	AF214350	AF214196
		<i>Parsonsia eucalyptophylla</i> F. Muell.	Australia, Queens- land	Forster 20118 (Z*)	AF214395	AF214241
		<i>Peltastes peltatus</i> (Vell.) Woodson	Paraguay, Alto Par- ana	Billiet & Jadin 3477 (Meise) 84-2849, Billiet 3526 (BR)	AF214396	AF214242
		<i>Pentalinon luteum</i> (L.) B. F. Hansen & R. P. Wunderlin	Australia, Brisbane	Forster 20089 (Z*)	AF214397	AF214243
		<i>Prestonia mollis</i> HBK	Puerto Rico	Struwe 1114 (NY)	AF214446	AF214292
		<i>Prestonia quinqueangularis</i> (Jacq.) Spreng.		Zona 833 (FTG), 94984 (FTG)	AF214410	AF214256
		<i>Funtumia elastica</i> (Preuss) Stapf		51-0287, Billiet 205 (BR)	AF214411	AF214257
Malouetieae			Congo, Eala	Couteaux s.n. 37-3045, Billiet 3126 (BR)	AF214357	AF214203
		<i>Holarrhena pubescens</i> H. P. Wall. & G. Don		Tavakali 1478 (MO)	AF214366	AF214212
		<i>Mascarenhasia arborescens</i> A. DC.		Meagher 998 (FTG) 4376 (FTG)	AF214378	AF214224

Appendix 1. Continued.

Subfamily	Tribe	Apocynaceae s.l.	Country	Voucher	GenBank Sequence	
					trnL	trnL-F
Mesechiteae		<i>Galactophora crassifolia</i> (Muell.-Arg.) Woodson	Brazil, Jacobina	Romero 1629 (NY)	AF214358	AF214204
		<i>Mandevilla</i> sp.	West Africa, Abou-abou	Amorium 1806 (NY)	AF214376	AF214222
Wrightieae		<i>Isonema smeathmanni</i> Roem. & Schult.	West Africa, Abou-abou	Oldeman s.n. (WAG) 1964-0527, Billiet 119 (BR)	AF214370	AF214216
		<i>Nerium oleander</i> L.	Tropical Africa	Potgieter 255 (NY)	AF214386	AF214232
Asclepiadoideae	Asclepiadeae	<i>Strophanthus gratus</i> Franch.	U.S.A.	850451 (CONN)	AF214430	AF214276
		<i>Wrightia tomentosa</i> Roem. & Schult.	U.S.A.	Gillis 10903 (FTG), 57157 (FTG)	AF214453	AF214299
		<i>Asclepias curassavica</i> L.	U.S.A.	Potgieter & Cahilly 243 (NY)	AF102381	AF214156
		<i>Asclepias syriaca</i> L.	U.S.A.	Potgieter & Cahilly 241 (NY)	AF214311	AF214157
		<i>Asclepias tuberosa</i> L.	Puerto Rico	Potgieter & Cahilly 242 (NY)	AF214312	AF214158
		<i>Calotropis procera</i> (Aiton) W. T. Aiton	Venezuela, Amazonas	Struwe 1095 (NY)	AF214324	AF214170
		<i>Cynanchum</i> sp.		72-2017 Billiet 2320 (BR)	AF214342	AF214188
		<i>Ditassa</i> sp.		Maguire 28465 (NY)	AF102412	AF214190
		<i>Gomphocarpus physocarpus</i> E. Mey.		P1965-5572 (C)	AF214360	AF214206
		<i>Gomphocarpus</i> sp.		Struwe & Albert 1194 (NY)	AF214361	AF214207
<i>Gonolobus patens</i> Decne.		Schmidt 2034 (MO)	AF214362	AF214208		
<i>Matelea</i> sp.		Taylor 11166 (MO)	AF214379	AF214225		
<i>Oxystelma bornouense</i> R. Br.		Schmidt 1724 (MO)	AF214392	AF214238		
<i>Pachycarpus</i> sp.		Potgieter 257 (NY)	AF214393	AF214239		
<i>Philibertia gilliesii</i> Hook. & Arn.		s1558-2389 (C)	AF214401	AF214247		
<i>Sarcostemma mulanjense</i> S. Liede & U. Meve		32AS13 (GA)	AF214418	AF214264		
<i>Sarcostemma stolonifera</i> B. R. Adams & R. W. K. Holland		32AS14 (GA)	AF214419	AF214265		
<i>Tweedia coerulea</i> D. Don ex Sweet		34512 3451-2 (C)	AF214443	AF214289		
<i>Tylophora sylvatica</i> Decne.		Stevens 3752 (MO)	AF214444	AF214290		
<i>Vincetoxicum hirsutinaria</i> Medic.		Endress s.n. (Z*)	AF214450	AF214296		
<i>Vincetoxicum nigrum</i> (L.) Moench		Potgieter 256 (NY)	AF214451	AF214297		
<i>Caralluma crenulata</i> Wall.		p1984-5290-3523 (C)	AF214325	AF214171		
<i>Caralluma gerstneri</i> Letty		1992-0097-80 (BR)	AF214391	AF214237		
<i>Ceropegia sandersoni</i> Decne. ex Hook.		Potgieter 248 (NY)	AF214333	AF214179		
<i>Duvalia elegans</i> (Masson) Haw.		1979-0578, EtOH (BR)	AF214347	AF214193		
<i>Duvaliandra dioscoridis</i> (Lavranos) M. G. Gilbert		1993-0034-26, EtOH (BR)	AF214346	AF214192		
<i>Echidnopsis cereiformis</i> Hook.		Potgieter 253 (NY)	AF214348	AF214194		
<i>Echidnopsis dammanniana</i> Sprenger		Bamps s.n., 78-0098, EtOH (BR)	AF214349	AF214195		

Appendix 1. Continued.

Subfamily	Tribe	Apocynaceae s.l.	Country	Voucher	GenBank Sequence	
					<i>trnL</i>	<i>trnL-F</i>
		<i>Frerea indica</i> Dalzell		p1995-5118 3523B (C)	AF214356	AF214202
		<i>Huernia keniensis</i> R. E. Fries	Kenya	852628 (CONN)	AF214368	AF214214
		<i>Ophionella arcuata</i> (N. E. Br.) Bruyns var. <i>mirkinii</i> (Pillans) Bruyns	South Africa	1987-0433, EtOH (BR)	AF214388	AF214234
		<i>Orbea variegata</i> (L.) Haw.	South Africa	19852630 (CONN)	AF214390	AF214236
		<i>Orbeanthus hardyi</i> (R. A. Dyer) L. C. Leach		p1980-5518-3524N-1 (C)	AF214389	AF214235
		<i>Piранthus geminatus</i> (Masson) N. E. Br.		<i>Camboulie s.n.</i> 1979-0712 (BR)	AF214402	AF214248
		<i>Stapelia leendertziae</i> N. E. Br.		p-1994-5328 3526 (C)	AF214403	AF214249
		<i>Stapelia peglere</i> N. E. Br.		<i>Gilbert s.n.</i> (NY)	AF214424	AF214270
		<i>Stapelia revoluta</i> Masson		32AS16 (GA)	AF212483	AF214271
		<i>Stapelia schinzii</i> Berger & Schltr.		<i>Potgieter 246</i> (NY)	AF214426	AF214272
		<i>Tavaresia grandiflora</i> (K. Schum.) Berger		p1980-5515-3524 (C)	AF214427	AF214273
		<i>Trichocaulon triebneri</i> Nel		p1990-5312 (C)	AF214433	AF214279
		<i>Tridentea longipes</i> (C. A. Lückh.) Leach	South Africa,	890032 (CONN)	AF214440	AF214286
			Richtersveld	1992-0113-96 (BR)	AF214441	AF214287
		<i>Tromotriche revoluta</i> (L.) Haw.		p-1980-5514-3524L-1 (C)	AF214442	AF214288
		<i>Dischidia bengalensis</i> Colebr.		920392 (CONN)	AF214343	AF214189
		<i>Dregea sinensis</i> Hemsl.		p-1972-5680 (C)	AF214345	AF214191
		<i>Fockea cylindrica</i> R. A. Dyer		p1994-5151 (C)	AF214352	AF214198
		<i>Fockea edulis</i> K. Schum.		<i>Potgieter 249</i> (NY)	AF214353	AF214199
		<i>Fockea sinuata</i> (E. Mey.) Druce		<i>Bourdoux s.n.</i> 1980-0551, EtOH (BR)	AF214354	AF214200
		<i>Hoya australis</i> R. Br. ex Traill		<i>Potgieter 247</i> (NY)	AF214367	AF214213
		<i>Marsdenia amorimii</i> G. Morillo	Brazil, Bahia	<i>Amorim 911</i> (NY)	AF214377	AF214223
		<i>Micholitzia obcordata</i> N. E. Br.	Thailand	<i>Seidenfaden 1972-5599</i> (C)	AF214381	AF214227
		<i>Telosma cordata</i> (Burm. f.) Merr.		<i>Gilding s.n.</i> (NY)	AF102493	AF214280
		<i>Cryptolepis oblongifolia</i> (Meisn.) Schltr.	Africa	<i>Albers 543</i> (MO)	AF214422	AF214268
		<i>Cryptostegia grandiflora</i> R. Br.	U.S.A., Hawaii	<i>Struве & Albert 1217</i> (NY)	AF214340	AF214186
		<i>Ischnolepis natalensis</i> (Schltr.) Venter		77BI306 (AAU, Z*)	AF214400	AF214246
		<i>Mondia whiteii</i> Skeels		<i>Potgieter 258</i> (NY)	AF214384	AF214230
				2717/94A (NY)		

Appendix 1. Continued.

Subfamily	Tribe	Apocynaceae s.l.	Country	Voucher	GenBank Sequence	
					trnL	trnL-F
Rauvolfioideae	Alstonieae	<i>Periploca graeca</i> L.		<i>Endress s.n. (Z*)</i>	AF102468	AF214244
		<i>Stomatostemma monteiroae</i> N. E. Br.		<i>Potgieter 257 (NY), 1320/93A (NY)</i>	AF214428	AF214274
		<i>Alstonia boonei</i> De Wild.	Ivory Coast	<i>Leeuwenberg 10724 (WAG)</i>	AF102374	AF214151
		<i>Aspidosperma australe</i> Müll. Arg.	Bolivia	<i>Jardim s.n. (MO)</i>	AF214313	AF214159
		<i>Aspidosperma cuspa</i> (Kunth) Blake	Colombia, Bolivar	<i>Gentry 47406 (NY)</i>	AF214315	AF214161
		<i>Aspidosperma desmanthum</i> Benth. ex Müll. Arg.	Belize, Orange Walk	<i>Whiteford 8023 (MO)</i>	AF214314	AF214160
		<i>Aspidosperma megalocarpon</i> Müll. Arg.	Mexico	<i>Breedlove 50932 (MO)</i>	AF102382	AF214162
		<i>Aspidosperma myristicifolium</i> (Markgraf) Woodson	Costa Rica	<i>Aguilar & Potgieter 3555 (MO)</i>	AF214317	AF214163
		<i>Aspidosperma pyriforme</i> Mart.	Bolivia	<i>de Queiroz 4136 (NY)</i>	AF214318	AF214164
		<i>Aspidosperma quebracho-blanco</i> Schtdl.	Bolivia, Santa Cruz	<i>Bettella 165 (MO)</i>	AF214319	AF214165
		<i>Geissospermum laeve</i> Miers	Brazil, Linhares	<i>Farias 23 (CVRD, Z*)</i>	AF214359	AF214205
		<i>Haplophyton crooksii</i> (L. D. Benson) L. D. Benson	U.S.A., Arizona	<i>Gilbertson s.n. (Z*)</i>	AF214363	AF214209
		<i>Laxoplumeria baehiana</i> Monach.	French Guiana, Région de Saül	<i>Mori 20933 (NY)</i>	AF214373	AF214219
		Alyxieae		<i>Microplumeria anomala</i> (Müll.-Arg.) Markgraf	Brazil, Amazonas	<i>Zarucchi 3116 (NY)</i>
<i>Strempelopsis strempeloides</i> Benth.	Cuba, Sanctus Es- piritus			<i>Acevedo-Rodriguez 6444 (NY)</i>	AF214429	AF214275
<i>Tonduzia longifolia</i> (DC.) Markgraf	Costa Rica, San Jose			<i>Morales 2776 (NY)</i>	AF214437	AF214283
<i>Tonduzia stenophylla</i> (Donn. Sm.) Pittier	Guatemala, Izabal			<i>Marshall 267 (NY)</i>	AF214438	AF214284
<i>Vallesia antillana</i> Woodson				<i>Zona 710 (FTG)</i>	AF214447	AF214293
<i>Alyxia buxifolia</i> R. Br.				s1981-0824-3393-2 (C)	AF214306	AF214152
<i>Condylocarpon amazonicum</i> (Markgraf) Ducke	French Guiana, Région de Saül			<i>Mori 20860 (NY)</i>	AF214337	AF214183
<i>Condylocarpon</i> sp.	Brazil, Ilheus			<i>Thomas 9956 (NY)</i>	AF214338	AF214184
<i>Lepiniopsis ternatensis</i> Valetton	U.S.A., Caroline Island			<i>Kanehira 566 (NY)</i>	AF214374	AF214220
<i>Plectanicia stenophylla</i> Jum.	Madagascar			<i>H. Petignat (Z*)</i>	AF214405	AF214251
<i>Pteralyxia kauaiensis</i> Caum	U.S.A., Hawaii			<i>Lorence 7768 (NY, Z*)</i>	AF214406	AF214252
					AF214412	AF214258

Appendix I. Continued.

Subfamily	Tribe	Apocynaceae s.l.	Country	Voucher	GenBank Sequence	
					trnL	trnL-F
Carisseeae		<i>Acokanthera oppositifolia</i> (Lam.) Codd	South Africa, Eastern Province	Bayliss 544 (NY)	AF214302	AF214148
		<i>Acokanthera rotundata</i> (Codd) Kupicha	Spain, Canary Islands	Wiese s.n. (WAG) 80-0032, Billiet 1875 (BR)	AF214303	AF214149
		<i>Carissa bispinosa</i> (L.) Desf. ex Brenan		1953-3766, Billiet 3529 (BR)	AF102391	AF214172
		<i>Carissa carandas</i> L.		77780 (FTG)	AF214327	AF214173
		<i>Hunteria umbellata</i> Hallier f.		Endress 97-16 (Z)	AF214369	AF214215
		<i>Picralima nitida</i> (Stapf) T. Durand & H. Durand	D. R. Congo	Kiss s.n. 86-0334, Billiet 3440 (BR)	AF214404	AF214250
		<i>Pleiocarpa mutica</i> Benth.		19610253, Billiet 1766 (Z*, BR)	AF214407	AF214253
		<i>Melodinus monogynus</i> Roxb.		1939-1959, Billiet 3359 (Z*, BR)	AF214380	AF214226
		<i>Allamanda</i> sp.		Wurdack s.n. (NY-1956)	AF214304	AF214150
		<i>Cerbera manghas</i> L.	U.S.A., Hawaii	Endress (s.n.) (Z*)	AF214330	AF214176
		<i>Cerbera odollam</i> Gaertn.	U.S.A., Hawaii	Struwe & Albert 1216 (NY)	AF214331	AF214177
		<i>Cerberiopsis candelabra</i> Vieill.	New Caledonia, Noumea	Tanguay 3288 (NOU)	AF102395	AF214178
		<i>Himatanthus</i> sp.	Brazil, Acre	Silveira 1336 (NY)	AF214364	AF214210
		<i>Himatanthus tarapotensis</i> (K. Schum. ex Markgr.) Plumel	Brazil, Acre	Cid 10241 (NY)	AF214365	AF214211
		Melodineae		<i>Plumeria alba</i> Kunth	Puerto Rico	Struwe 1096 (NY)
<i>Plumeria obtusa</i> L.	Guatemala			Specht s.n. (NY)	AF214409	AF214255
<i>Skytanthus acutus</i> Meyen	Chile			Grosjean s.n. (Z*)	AF214423	AF214269
<i>Thevetia ahouai</i> (L.) A. DC.				Zona 618 (FTG, Z*), 95-529 (FTG)	AF214435	AF214281
<i>Thevetia peruviana</i> (Pers.) K. Schum.				Watson 1473 (FTG), 81393 (FTG)	AF214328	AF214174
				Struwe & Albert 1213 (NY)		
				Phillippe 26927 (NY)	AF214436	AF214282
Tabernaemontaneae		<i>Bonafousia siphilitica</i> (L. f.) L. Allorge	U.S.A., Hawaii		AF214322	AF214168
		<i>Callichilia barteri</i> (Hook. f.) Stapf	French Guiana, Région de Saül	Van Stellen 116 (WAG) 1982-0466, Billiet 3527 (BR)	AF214323	AF214169
		<i>Macoubea guianensis</i> Aubl.	Brazil, Linhares	Folli 5 (CVRD, Z)	AF214375	AF214221
		<i>Molongum laxum</i> (Benth.) Pichon	Brazil, Amazonas	Rodrigues 10741 (NY)	AF214383	AF214229
		<i>Tabernaemontana citrifolia</i> L.	Puerto Rico, Rio Grande	Taylor 11858 (MO)	AF214431	AF214277
		<i>Tabernaemontana floribunda</i> Blume	U.S.A., Florida	Fantz 3161 (FTG) 591079 (FTG)	AF214351	AF214197

Appendix 1. Continued.

Subfamily	Tribe	Apocynaceae s.l.	Country	Voucher	GenBank Sequence	
					trnL	trnL-F
Vinceae		<i>Tabernaemontana</i> Plum.	Brazil, Acre	Daly 603 (NY)	AF214399	AF214245
		<i>Tabernanthe iboga</i> Baill.		1981-0308, Billiet 3557 (BR, Z*)	AF214432	AF214278
		<i>Voacanga africana</i> Stapf	Ivory Coast, Sasandra	Leeuwenberg 12054 (WAG)	AF214452	AF214298
		<i>Amsonia orientalis</i> Decne.		80-0066, Billiet 3528 (BR)		
		<i>Amsonia tabernaemontana</i> Walter	U.S.A.	3403 (C)	AF214416	AF214262
		<i>Catharanthus roseus</i> G. Don		Potgieter & Cahilly 237 (NY)	AF214307	AF214153
		<i>Neisosperma nakiana</i> (Koidzumi) F. R. Fosberg & M.-H. Sacht		Potgieter 245 (NY)	AF102392	AF214175
		<i>Ochrosia elliptica</i> Labill.		Endress 884 (Z*)	AF214385	AF214231
				Gillis 7573 (FTG, Z*)	AF214387	AF214233
				X-2-101 (FTG)		
		<i>Rauwolfia balansae</i> (Baill.) Boit.	New Caledonia	Lowry 4760 (MO)	AF214413	AF214259
		<i>Rauwolfia serpentina</i> Benth. ex Kurz		Potgieter 252 (NY)	AF214414	AF214260
					AF214415	AF214261
Willughbeeae		<i>Vinca difformis</i> Pourr.		(DAV)	AF214448	AF214294
		<i>Vinca minor</i> L.		Potgieter 256 (NY)	AF214449	AF214295
		<i>Couma macrocarpa</i> Barb. Rodr.	Colombia	Cárdenas 7379 (Z)	AF214339	AF214185
		<i>Lacmellea aculeata</i> (Ducke) Monachino	Brazil, Bahia	Amorim 1229 (NY)	AF214371	AF214217
		<i>Landolphia incerta</i> (K. Schum.) Persoon	Eastern Ghana	Schmidt 1696 (MO)	AF214372	AF214218
		<i>Pacouria guianensis</i> Aubl.	French Guiana, Région de Saül	Allorge 337 (NY)	AF214394	AF214240
		<i>Saba comorensis</i> (Bojer) Pichon		00-5412, Billiet 3442 (BR)	AF214417	AF214263
		<i>Secamone glaberrima</i> K. Schum.	Madagascar, Toocmasina	Stevens 26007 (MO)	AF214420	AF214266
		<i>Secamone oleaeifolia</i> Decne.	Madagascar, Antananarivo	Stevens 26005 (MO)	AF214421	AF214267
Secamonoideae						

DIVERSITY AND RELATIONSHIPS WITHIN THE PERIPLOCOIDEAE (APOCYNACEAE)¹

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Rudolf L. Verhoeven²

ABSTRACT

Morphological diversity and phylogenetic relationships in the Periplocoideae are analyzed. Most of the species of this subfamily inhabit tropical or subtropical forests and savannas as woody climbers, woody shrubs, epiphytes, or herbaceous geophytes. The flower is complex and has evolved into many forms, always incorporating a corolline corona, interstaminal corona-like nectaries, and stamens fused with the style-head into a gynostegium. Pollen is borne in tetrads, less often as pollinia, which are shed onto spatulate translators that are secreted in grooves around the periphery of the style-head. Phylogenetic relationships among the Periplocoideae genera are analyzed cladistically using morphological characters, mainly of the flower. This analysis reveals one larger clade and two smaller clades. Eleven new generic synonyms, six new specific synonyms, and fifteen new species combinations are required within the subfamily. A key to the genera of the Periplocoideae is included.

Key words: Apocynaceae, generic key, morphological diversity, nomenclatural corrections, Periplocoideae, phylogeny.

The Periplocoideae (Apocynaceae) are restricted to the Old World, in Africa, Madagascar, Europe, Asia, and Australia, mainly in tropical and subtropical regions, with the majority of the species found in tropical evergreen rainforest, tropical seasonal (monsoon) forest, and tropical woodlands (savannas) (Venter et al., 1990a; Venter, 1997; Venter & Verhoeven, 1996a). A small number of taxa, e.g., *Raphionacme galpinii* Schltr. and *R. hirsuta* (E. Mey.) R. A. Dyer, inhabit the grasslands, and a few taxa are found in desert and semi-desert habitats (e.g., the three species of *Ectadium* E. Mey., *Periploca aphylla* Decne., *P. visciformis* (Vatke) K. Schum., *Raphionacme haenelii* Venter & R. L. Verh., and *R. namibiana* Venter & R. L. Verh. (Venter, 1997; Venter & Verhoeven, 1986b, 1996b; Venter et al., 1990b)). Interestingly, no Periplocoideae inhabit the South African Cape Floral Kingdom with its winter rainfall. However, a few species, e.g., *Periploca angustifolia* Labill. and *P. gracilis* Boiss., occur in the Mediterranean macchia of North Africa and Europe (Venter, 1997).

At present the Periplocoideae comprise 31 genera and 181 species. Most of the genera comprise only a few species. The largest genera are *Raphionacme* Harv. (37 species), *Cryptolepis* R. Br. (27 spe-

cies), *Pentopetia* Decne. (21 species) (Klackenberg, 1999), and *Periploca* L. (13 species) (Venter, 1997). The difference between these numbers and the 44 genera and ca. 190 species given by Venter and Verhoeven (1997) in their classification of the subfamily is because a number of little known genera, most of them monotypic and from Asia, have been put into synonymy since then. Two genera have been placed in synonymy by Klackenberg (1997, 1998, 1999) and another 11 genera are synonymized here (one genus previously incorrectly synonymized and corrected here) (Appendices 2 & 3). During the same time 14 new species have been added (Klackenberg, 1997, 1998, 1999; Venter & Verhoeven, 1999, 2000), a number of existing ones have been placed in synonymy (Klackenberg 1997, 1998, 1999), and another 6 are synonymized in the present article (Appendix 3). Fifteen new combinations are validated herein (Appendix 3).

The majority of periplocoid taxa are woody climbers of moist or drier forests or savanna, such as *Buckollia* Venter & R. L. Verh. in Africa (Venter & Verhoeven, 1994b), *Cryptolepis* (in part) in Africa and Asia, and *Gymnanthera* R. Br. in Asia and Australia (Forster, 1991). Some of the climbers, e.g., *Mondia* Skeels and *Tacazzea* Decne., both

¹ The University of the Free State, Bloemfontein, and the National Research Foundation, Pretoria, are thanked for their financial support toward the revision of the Periplocoideae. We also extend thanks to the herbaria, mentioned under Materials and Methods, for the use of their specimens and libraries, to Rouvay Roodt, who carried out the cladistical analysis, and to Johan Spies for his comments on the resultant cladogram. We are deeply grateful to Mary Endress for her valuable comments and assistance.

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